GENETIC RELATIONS OF CHLOROPHYLL AND ANTHOCYANIN SEEDLING CHARACTERS IN MAIZE[1]

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INTRODUCTION

During the last decade almost a hundred genetic factors have been discovered in maize. These factors in most cases form interesting and interrelating series with one another. In particular the pericarp color, the aleurone color, the silk color, the anthocyanin color, and the seedling chlorophyll color series are the ones most commonly known. These involve many factors, some of which are found in two or more series.

One of the most interesting of the series of factors named above is that of seedling chlorophyll. This subject has been reviewed and discussed by LINDSTROM (1918, 1921).

Another prominent series is that of anthocyanin. The complicated genetic relations of this series have been discussed by EMERSON (1921).

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As we already have many of the facts of chlorophyll and anthocyanin inheritance at our command, it is desired to show in this report the genetic relations of the anthocyanin and the chlorophyll seedling characters in maize. Although the relations between the anthocyanin and the chlorophyll characters are shown by seedling characters, the factors, as far as the anthocyanin is concerned, are identical with those dealt with by EMERSON (1921) in his results on the mature-plant pigment types.

It was first desired to test the relation of each factor in the $A\,B\,P_1$ anthocyanin series (EMERSON 1921) with the w and v chlorophyll factors (LINDSTROM 1918) with special reference to possible linkage or independence. Results of this test have been obtained on all of these relations with the exception of the B with the w or the v factors. As the results indicate a placing of the W_1 and V_1 factors each in one of the linkage groups, it is not necessary to have their relation with the B factor at this time.

NOMENCLATURE AND DESCRIPTIONS

Red- and non-red-stem seedlings are terms which will be applied herein to the presence and absence, respectively, of anthocyanin pigment in the seedlings. This color generally develops more intensely on the first leaf sheath. On some seedlings it develops well up into the leaf, and in most red seedlings the tip is brightly colored. In some cases, the red develops first on the stem proper, while in others the color may develop first at the tip. In some red-stem seedlings only traces of red are found; these traces of red may be found at the tip, on the first leaf sheath, at the basal node, or on the stem proper. The presence of red shows the presence of the A factor. Non-red-stem seedlings are those seedlings having no trace of red that can be detected by the naked eye or even by the use of a hand lens.

A description of each of the factors concerned in obtaining these relations is given below:

W_1 w_1, a factor pair for green chlorophyll and white or albino seedlings (LINDSTROM 1918). The recessive condition has the effect of inhibiting the development of any chlorophyll pigment, and, of course, the seedlings carrying this factor die when the endosperm of the seed is used up.

W_2w_2, another factor pair for albino seedlings. This factor pair has not heretofore been described. It first appeared in material received by Doctor LINDSTROM from Doctor W. E. CASTLE. The characters are identical with those which are associated with the W_1w_1 factor pair and it is impossible to distinguish, phenotypically, seedling characters caused

by W_2w_2 from those caused by W_1w_1. It may be proved later that these are identical factors, but observations thus far indicate that they are two entirely different factor pairs. Further discussion of w_2, together with a discussion of a red- and a non-red-stem character with which the w_2 is linked, will be given in another section of this paper.

V_1v_1, a factor pair for green chlorophyll and the virescent character found in the seedling stage (LINDSTROM 1918). The v_1v_1 condition prevents the development of the green chlorophyll only for a time. When the seedlings first come up they have a slight trace of green at the tips of the leaves, then they gradually turn green and may even grow to maturity and produce ears.

A a, a factor pair for the development of anthocyanin pigment in the mature plant, in aleurone cells, pericarp, silks, etc. (EMERSON 1918, 1921; ANDERSON 1921).

EMERSON has shown the relations of this factor very definitely so far as mature-plant colors and aleurone are concerned, and his papers bring out the fact that those types having *A* present, develop red in the seedling stage, while those having *aa* are devoid of anthocyanin.

At the beginning of the work reported herein, a number of seedlings were grown from each ear in the several mature plant types available. Then, during the summer, plants were observed in the field to note possible segregation there. It was found that in the presence of *A* the seedlings were red while in the absence of *A* the seedlings were non-red.

B b, a factor pair for brown pigment in the mature plant and an intensifier of *A* (EMERSON 1921). EMERSON has pointed out that it is not possible to distinguish seedlings involving this factor pair; that is, he could not distinguish in the seedling stage the sun-reds ($A\ B\ p_l$) from the dilute-sun-reds, ($A\ b\ p_l$) which differ only in the *B* factor.[2] As far as the present observations go this has been the case, although families have been observed which segregate rather distinctly into intense-red stems and light-red stems. This has occurred in sun-red families and although the two classes can not be absolutely differentiated, fairly definite classes can be made. Whether this is due to the presence and absence of the *B* factor remains to be tested. While this has been found in some families there are other families in which dilute-sun-red seedlings are just as intense if not more so than seedlings from sun-red plants.

$P_l\ p_l$, a factor pair for purple anthocyanin pigment in the mature plant (EMERSON 1921). This factor pair occurs in the same linkage group with

[2] An exception to this occurs when R_g or r^g is homozygous in progenies heterozygous for the *B* factor (EMERSON and HUTCHISON 1921).

Y y, a factor pair for yellow and white endosperm, and S_m s_m a factor pair responsible for salmon-colored silks (ANDERSON 1921).

Y y, a factor pair for yellow and white endosperm (EAST and HAYES 1911; EMERSON 1921). As previously stated this factor pair occurs in the same linkage group as the P_l p_l and S_m s_m factor pairs (EMERSON 1921, ANDERSON 1921).

$r^g r^g$, a factor pair wherein the recessive r^g r^g, when *B* is not present, prevents the expression of *A* in the foliage. $R^g R^g$, $R^g r^g$, and $r^g R^g$ will apply the same as $r^g r^g$ (EMERSON 1921).

MATERIAL

The work herein reported was started in the spring of 1920 at the UNIVERSITY OF WISCONSIN, with material kindly furnished by Doctor E. W. LINDSTROM. Some of this material originally came from Doctor W. E. CASTLE of HARVARD UNIVERSITY, while some F_1's came from Doctor E. G. ANDERSON, then of CORNELL UNIVERSITY. All the work reported here was carried on in conjunction with the Genetics Department of the UNIVERSITY OF WISCONSIN.

The writer wishes to express his sincere gratitude to Doctor LINDSTROM, who, at all times throughout these studies, not only put his material at the disposal of the writer, but also tendered valuable aid, encouragement, inspiration and advice; and by such generosity and aid made possible these studies.

Grateful acknowledgment is made also to Prof. L. J. COLE, for encouragement and criticisms.

Some of the data were taken directly from the records of Doctor LINDSTROM and wherever this was done reference will be noted in this paper. The source of the material will be designated by prefixing to the pedigree number the letter "L" when from Doctor LINDSTROM, and "A" when the material is from Doctor ANDERSON.

METHODS

Individual notes were taken at flowering time on all the material.

In calculating closeness of fit, the method reported by ELDERTON (1901) was used. The crossover values were calculated by HALDANE'S method for the determination of percentage of crossing over from the F_2 population (HALDANE 1919).

Wherever it was necessary to use the probable-error method, the general formula, P. E. $= .6745 \sqrt{p \times q \times n}$ was used, the letters p and q being

the elements of the ratio, and n the number of individuals in the population.

DATA AND RESULTS

In the preceding sections of this paper the characters to be discussed are given along with their specific factors. The source of material and the methods used in these studies, also, are given.

In the following pages are found the data and result of the genetic relations of the chlorophyll and the anthocyanin types.

It is desired to show, first, the relation of the W_1 factor with the $A\ B\ P_l$ factor series. Then, to show the relation of the V factor with the same anthocyanin series, and, finally, to show the relation of W_2, another factor for albino seedlings, with an anthocyanin factor where this anthocyanin factor is not known, thus far, with certainty.

INDEPENDENT RELATIONSHIP OF THE FACTOR PAIRS W_1w_1 AND Aa

Before the linkage relations of W_1w_1 and Yy and V_1v_1 and Aa were obtained, many data had accumulated on the relationship of the W_1w_1 and Aa factor pairs. Most of these data came from self-pollinating dilute-sun-reds in families which were segregating for dilute-sun-reds, $A\ b\ p_l$, and full greens, $a\ b\ p_l$. The fact that they were segregating for dilute-sun-red and green or non-red was enough to show that within such families two-thirds of all dilute-sun-reds would be heterozygous for the A factor or of the composition $Aa\ bb\ p_lp_l$. The testing of W_1w_1 and Aa was accomplished in the greenhouse with seedling characters by planting the seed of dilute-sun-reds. It was found that non-red seedlings were produced in the greenhouse in the absence of A. That is to say, all full greens or non-reds gave only non-red-stem seedlings the following winter in the greenhouse, while among dilute-sun-reds red-stem seedlings were found.

Progenies totaling 114 were tested for the relation of these two factor pairs. The total number of seedlings counted in obtaining this relation amounted to 9736.

The sum of all the seedling counts of those progenies segregating for the four classes of characters, namely, green, red stem, green non-red stem, white red stem, and white non-red stem, or factorially W_1A, W_1a, w_1A, w_1a, respectively, are shown in table 1.

It is obvious that the above relation of calculated to observed does not give a good fit when all four classes are taken into consideration. It is noticed that the poorness of fit appears in the green chlorophyll classes.

The non-red-stem class is considerably higher and the red-stem class is lower than the expected. This is due no doubt to the fact that within the greens the classification of red- and non-red-stems is sometimes difficult. The red, although it may be present, may pass unnoticed, being

TABLE 1

	GREENS		WHITES		TOTAL
	Red-stem W_1A	Non-red-stem W_1a	Red-stem w_1A	Non-red-stem w_1a	
Observed	5364	1930	1842	600	9736
Calculated, 9 : 3 : 3 : 1	*5476*	*1826*	*1826*	*608*	*9736*
o−c	−112	+104	+16	−8	

P=0.038

masked by the green. A few such errors in 99 progenies would be more than enough to account for the poorness of fit within these two classes.

Taking the relation of the red- and non-red-stems within the white class only, we have the situation shown in table 2.

TABLE 2

	RED-STEM	NON-RED-STEM	TOTAL
Observed	1842	600	2442
Calculated, 3 : 1 ratio	*1832*	*610*	*2442*

$$\frac{\text{Dev.}}{\text{P.E.}} = \frac{10.0}{14.7} = 0.69$$

Here the deviation divided by the probable error is less than one, which shows an excellent agreement of theoretical and observed facts.

The progeny test was computed for the four genotypic classes within the phenotype W_1A class, namely, W_1W_1AA, W_1W_1Aa, W_1w_1AA, W_1w_1Aa. The reason for this was that the whites w_1w_1 always die in the seedling stage and the full greens (a a) were discriminated against in the field due to the fact that they were known not to give the desired four-class segregation.

The results of the progeny test are as given in table 3. The fit here is very poor. The chances are only 1 : 22 that the deviations here are due to random sampling.

It is possible that W_1 and A may be loosely linked. If this were the case some of these progenies would show repulsion and others coupling.

Therefore in order to test this possibility the percentage recombination was figured on *each* progeny under consideration, which was segregating for the four classes. Attention is called to the fact that these calculations were made on F_3 progenies and the parental combinations were not known. Accordingly, the crossover classes are shown by calculating the percentage of recombination by the use of HALDANE'S method, as noted in a previous

TABLE 3

RATIO	GENOTYPES	OBSERVED	CALCULATED	o−c	(o−c)²	$\frac{(o-c)^2}{c}$
1	W_1W_1AA	10	13	3	9	0.69
2	W_1W_1Aa	18	25	7	49	1.96
2	W_1w_1AA	22	25	3	9	0.36
4	W_1w_1Aa	64	51	13	169	3.31
9	Total	114				6.32

$\chi^2=6.32$; $P=0.044$.

section of this paper. In using this method the results show directly whether the factors are together in form of coupling or in the form of repulsion. The curve showing the distribution of the percentages of recombination is given in figure 1, as also is the mean and standard

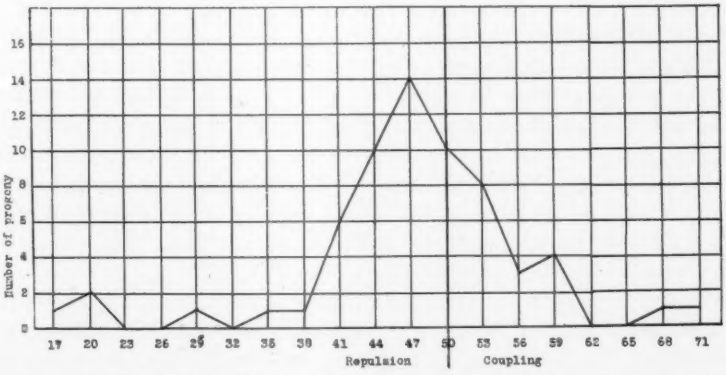

FIGURE 1.—Curve showing frequency of the percentage crossing over in 63 progenies which were heterozygous for *W* and *A*. Mean, 47.1±.80; standard deviation, 9.38±.84; coefficient of variability, 19.9±.01.

deviation. This curve is one of normal frequency, and although it has a wide distribution it seems as though W_1 and *A* are independent of each other. Nevertheless, it is possible from these data, alone, that W_1 and

A might be in the same linkage group but about 47 units apart. At any rate, if there is a linkage here it still remains unproven.

LINKAGE INVOLVING THE W_1w_1 AND P_lp_l FACTOR PAIRS

In order to test the P_lp_l factor pair with W_1w_1 and V_1v_1, plantings of ears heterozygous for P_l and W_1, as well as heterozygous for P_l and V_1 were made in the dark-room. When planted in the light the kernels from the ears used in this dark-room gave only red-stem seedlings, and as far as the writer could see all the red stems were of the same intensity, that is, no definite classes of different intensities could be made.

The seedlings were grown first in the dark in order to allow the anthocyanin produced by the P_l factor to develop, then those seedlings showing red were marked and placed in the light so as to determine the chlorophyll characters. The purple plant pigment develops in the absence of light as well as in the presence of light (EMERSON 1921). The other anthocyanin pigments of the *A B* P_l series are produced only in the presence of light. For instance the sun-red *A B* p_l, and dilute-sun-red type *A b* p_l develop in the light only, while purples and dilute-purples, *A B* P_l and *A b* P_l, respectively, develop some anthocyanin in the dark (EMERSON 1921). This was also found to be the case with seedling characters. The different plant-color types were grown in the dark as a check and the only ones to develop any color in the absence of light were those having *A* P_l factors. Accordingly, this development of anthocyanin in the dark was attributed to the P_l factor.

The numbers which show the linkage of P_l and W_1 are very small. Doctor LINDSTROM'S cultures which produced whites, practically all lacked the P_l factor. In one cross of dilute-purple, (*A b* P_l) W_1W_1, with a sun-red, (*A B* p_l) W_1w_1 three ears were found in F_2 which segregated for both P_lp_l and the W_1w_1 factor pairs. From another cross, a dilute-sun-red, (*A b* p_l) W_1w_1, with a purple, (*A B* P_l) W_1W_1, one F_2 ear also was found to segregate for the two factor pairs, $W_1w_1P_lp_l$.

In the dark-room test it was impossible to obtain four classes, as the classification between red- and non-red-stem in the dark was somewhat uncertain. It probably takes longer for some individuals to develop red than it takes others. At least, the red does not develop very uniformly, and as the seedlings cannot be left in the dark for more than a week, it is possible that some of the seedlings carrying the P_l factor do not develop the red in this length of time. But, according to the law of chance, one probably should get the same ratio of normal greens to whites among those found with red in the dark at this time, as if one could have obtained

all the individuals carrying the P_1 factor. The seedlings cannot be left in the dark for much over a week for the reason that they become so rank in growth that it is nearly impossible to handle them; also, if left too long in the dark their vigor is impaired, and it is difficult for those which are genetically green to normally develop the chlorophyll.

The four ears mentioned in the preceding paragraph, all of which were of the genetic composition $W_1w_1\ P_1p_1$, were grown in the dark and the data which were obtained are presented in table 4.

TABLE 4

PEDIGREE NUMBER	SEEDLING TYPE AS TO CHLOROPHYLL (ALL RED-STEMMED IN THE DARK)		TOTAL
	Green	White	
361–2	110	22	
361–7	74	14	
361–10	82	10	
366–2	24	2	
Total.....	290	48	338
Calculated, 3 : 1....	*254*	*84*	*338*
Deviation.....	36	36	

Dev.÷P. E.=6.6

Obviously this is not a normal, independent relationship, since deviation as great as this would be expected to occur only once in about one-fourth of a million trials.

Now, if these two factors do not segregate independently, have they a linkage value?

The results of the above data fitted to 25 percent crossing over are as shown in table 5.

TABLE 5

	SEEDLING TYPE AS TO CHLOROPHYLL (ALL RED-STEMMED IN THE DARK)		TOTAL
	Green	White	
Observed.....	290	48	338
Calculated, 25 percent crossing over..	*289*	*49*	*338*

Dev.÷P. E.=1÷4.37=0.2

Evidently the data show approximately 25 percent crossing over, and since they fail to give a good fit for independent inheritance, it seems as

if these data are worth considering very seriously as indicating a fairly close linkage relationship between the W_1 and P_l factors.

A field test was made in 1922 to check these data. This test consisted of planting F_2 seed from the same ears as those used in the dark-room test. The white or albino seedlings were removed and at maturity notes were taken on the greens as to their type of anthocyanin.

The results are given in table 6.

TABLE 6

	GREENS	
	P_l	p_l
Observed	61	6
Calculated, 25 percent crossing over	*57*	*10*
Deviation	4	4

Dev. ÷ P. E. = 1.67

The data here show less than 25 percent crossing over and therefore support the hypothesis that W_1 and P_l are closely linked. Data follow which will substantiate the results obtained here.

LINKAGE OF W_1w_1 AND Yy FACTORS

Realizing that the dark-room test was not an absolute test, especially since the numbers of plants were small, it was deemed necessary to test the relationship of the P_l and W_1 by other means. A good test would be one in which another factor lying in the same linkage group with P_l was involved. Indications of such a linkage became apparent when data involving Yy and W_1w_1 were scrutinized carefully. Some earlier work by KEMPTON seems to be best explained on this basis.

KEMPTON (1907) pointed out a correlation between endosperm color and chlorophyll seedling color. From a cross between *Zea tunicata* and *Zea ramosa*, one ear was obtained from selfing an F_1 plant which segregated for endosperm color and green and albino seedlings. His results are given in table 7.

TABLE 7

	NORMAL	ALBINO	TOTAL
Yellow seeds	57	13	70
White seeds	6	12	18
Total	63	25	88

It seems clear from the data pointed out in the succeeding paragraphs that KEMPTON was dealing with W_1w_1Yy factor pairs. This linkage was not known to be related to the W_1w_1 factor pair in question until the W_1w_1 and P_l linkage was observed.

It was shown that the Yy factor pair for yellow and white endosperm is linked with the P_lp_l factor pair for purple anthocyanin plant pigment (EMERSON 1921). It is understood that there are two or more independent factor pairs for the presence of yellow and white endosperm. Accordingly, if P_l and W_1 are linked, one of the yellow and white endosperm factors (Yy) is linked with W_1, the others should be independent.

The factor pair for yellow and white endosperm dealt with here is presumably the same (Yy) as that reported and used by EMERSON (1921) and ANDERSON (1921), since no case was found in this work which shows independent inheritance between W_1 and Y.

The linkage data reported herein indicate about 42 percent of crossing over between Yy and W_1w_1. This linkage was found in several families, the first being the family L4144.

Family L4144 is the progeny of a cross, L3360-28 W_1W_1YY with L3349-7 W_1w_1yy, which was made and grown by Doctor LINDSTROM. The seedling counts were made by the writer. The distribution of the progeny of the heterozygous (W_1w_1) F_1 plants in the F_2 generation of this cross is shown in table 27 (see appendix).

A summary of the results of this cross is shown in table 8.

TABLE 8

	YELLOW SEED		WHITE SEED		TOTALS
	Green	White	Green	White	
Observed	727	209	223	116	1275
Calculated, 42 percent crossing over	*732*	*217*	*217*	*109*	*1275*

$\chi^2 = 0.943$

In another family, L3348, (F_3) which was turned over to me, the linkage of W_1 and Y was also found. LINDSTROM'S 3348 came from yellow seeds in a family segregating for yellow and white endosperm color. Some of the ears of L3348 were segregating for yellow and white endosperm and from these nine ears were obtained which were segregating for the four classes of characters concerned. This family had been inbred for three generations and came originally from a cross, L937-14×L812-1, made by Doctor LINDSTROM.

Within the progenies of this family it was expected that both the repulsion and coupling phases would occur, as some plants should have the arrangement $\widehat{YW_1}\ \widehat{yw_1}$ and others $\widehat{yW_1}\ \widehat{Yw_1}$, where both would segregate for the four classes of characters. The plants which contained the former would make up the coupling phase and those having the latter would produce the repulsion phase. In no case was there a doubt of the placing of a progeny in one phase or the other.

The progenies of this family are listed in table 28. The summary of results where both factors are involved is given in table 9.

TABLE 9

1. *Coupling phase: (progeny numbers L3348-4, 8, 10, 24, and 21)*

	YELLOW SEEDS		WHITE SEEDS		TOTAL	χ^2
	Green YW_1	White Yw_1	Green yW_1	White yw_1		
Observed	405	112	124	59	700	
Calculated, 42 percent crossing over	*402*	*119*	*119*	*60*	*700*	0.66
(o−c)	3	7	5	1		

2. *Repulsion phase: (progeny numbers L3348-14, 28 and 32)*

Observed	456	181	186	41	864	
Calculated, 42 percent crossing over	*460*	*184*	*184*	*37*	*865*	0.538
(o−c)	4	3	2	4		

The goodness of fit here is very significant, as it closely approaches perfection.

To test this family further, the progeny test was computed. The parent ear of L3348, namely, L2205-10, was segregating for the four characters under consideration, namely, yellow and white endosperm color and green and white chlorophyll seedling characters. The distribution of the classes in this parent ear is found at the bottom of table 28. The progeny test was calculated for the repulsion phase, as the parent ear, L2205-10, exhibited this phase, though not so distinctly as its progeny. There was a small deficiency of white seedlings from this ear and in order to check this in the next generation of this family, green and white seedlings of heterozygous individuals were totaled. The result of this showed a definite 3 : 1 relationship between the two.

In calculating the agreement of the progenies of the parent ear L2205-10 only the yellow seeds were used.

The results of the progeny test are as shown in table 10.

TABLE 10

GENOTYPES	EXPECTED RATIO FOR 42 PERCENT CROSSING OVER REPULSION PHASE	OBSERVED	CALCULATED
YYW_1W_1	1.0	2.0	2.4
YYW_1w_1	2.8	7.0	6.7
YyW_1W_1	2.8	5.0	6.7
YyW_1w_1	5.92	16.0	14.2
Total	12.52	30.0	30.0

$\chi^2 = 0.738$

The result of the progeny test further proved that the parent ear was of the constitution $\widehat{Yw_1}.\widehat{yW_1}$, since, when the calculated was computed with 42 percent crossing over between Y and w_1 and y and W_1, there was found to be a good agreement between calculated and observed, χ^2 being less than 1.

THE CHROMOSOME MAP

The 30 percent crossing over between Y and P_l was reported in 1921 (EMERSON 1921), as was also the 37 percent crossing over between Y and S_m, and the 10 percent crossing over between P_l and S_m (ANDERSON 1921). The relationship of W_1 in this group has been reported in a preceding section of this paper. Even though the 42 percent crossing over between W_1 and Y will have to be checked by determining its relation with other factors between these two points, there seems to be no doubt at this time that the W_1 factor should be placed in this group along with Y, P_l and S_m, and in the relative position shown in figure 2.

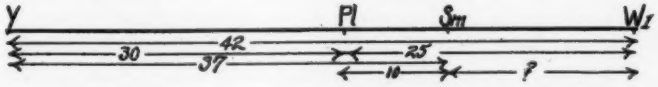

FIGURE 2.—The chromosome map with the relative distances plotted in percentages of crossing over.

Figure 2 gives the map of the chromosome under consideration as it now stands and as the work reported herein seems to indicate.

LINKAGE INVOLVING THE V_1v_1 AND Aa FACTOR PAIRS

The data gathered on the relation between these two factor pairs indicate a very loose linkage. Such a loose linkage is apt to be overlooked

because 45 percent crossing over, which apparently prevails, approaches independent inheritance.

Evidence of linkage between the Aa and V_1v_1 factor pairs was found in the progeny of three separate crosses of a dilute-purple plant, $\widehat{AV_1}\ \widehat{av_1}\ bb\ P_lp_l$, by a dilute-sun-red $\widehat{AV_1}\ \widehat{AV_1}\ bb\ p_lp_l$. One ear from each cross was obtained in F_2 which gave the coupling-phase segregation for the four seedling characters, namely, green red-stem, green non-red-stem, virescent red-stem, and virescent non-red-stem. The results are given in table 11.

TABLE 11

PEDIGREE NUMBER	GREENS		VIRESCENTS		TOTAL
	Red-stem V_1A	Non-red-stem V_1a	Red-stem v_1A	Non-red-stem v_1a	
368–8	271	89	86	40	
370–3	115	28	49	11	
373–4	161	44	53	23	
Observed total	547	161	188	74	970
Calculated, 45 percent crossing over	*558*	*170*	*170*	*72*	*970*

$\chi^2 = 2.64$ $P = 0.46$

Here, P indicates a good agreement between the observed and calculated on a basis of 45 percent crossing over, although these data also fit, fairly well, an independent segregation.

From a cross, A 1958 $\widehat{V_1a}\ \widehat{v_1A} \times$ A 1136-4 $\widehat{V_1a}\ \widehat{V_1a}$, three plants were self-pollinated. In the F_2 of this cross the mature plants showed the segregation of the Aa factor pair, although the ratio of 3 : 1 was not closely approximated. This was probably due to small numbers. In F_3 the segregation of the Aa factor pair gave a good 3 : 1 ratio.

Among the progenies from one F_2 family, seven were heterozygous for both factor pairs, and only two were found that were not heterozygous for both. In the other two F_2 families not a single F_3 progeny was found that showed a segregation for both factors. According to the F_3 data of the three families, noted above, there were five F_2 individuals having red stems and not segregating for the four classes, that is, not heterozygous for both factor pairs, or a ratio among those having red stems of five not segregating for the four classes to seven which did segregate for the four classes. The ratio expected with independent inheritance is 5 to 4 for all individuals of the AV_1 phenotype. Although this is not very close agree-

ment between expected and observed, with such small numbers we can say that such deviation might be expected occasionally with independent inheritance. The progeny test, however, verifies the hypothesis.

The three F_1 individuals selfed gave in F_2 the repulsion phase of linkage. In F_3, five out of seven progenies giving segregation for the four classes showed repulsion, the others exhibited the coupling phase.

The results of the F_2 and the F_3 repulsion phase are given in table 12 (progeny numbers from table 29, 54-3, 4, 5, and 371-7, 9, 10, 14 and 15).

TABLE 12

	GREENS		VIRESCENTS		TOTAL
	Red-stem	Non-red-stem	Red-stem	Non-red-stem	
Observed	692	189	227	52	1160
Calculated, 45 percent crossing over	*640*	*230*	*230*	*60*	*1160*

For the four classes a close agreement with theory is impossible due to the fact that there is an excess of red-stemmed individuals especially within the green chlorophyll classes. Under some conditions it is very difficult to classify non-red and red-stem seedlings. Especially is this true within the green classes. Generally speaking it is comparatively easy to classify stem color within the virescent classes. Considering the two virescent classes alone, on a ratio of 3.84 to 1, the ratio expected for 45 percent crossing over (of virescent red-stem to virescent non-red-stem) the deviation is 6; the P. E. = 4.9. Accordingly, deviation divided by probable error is $6 \div 4.9 = 1.22$, which shows a fairly close agreement in these two classes.

The results of the coupling phase in F_3 are given in table 13 (progeny numbers from table 29, 371-3; 371-13).

TABLE 13

	GREENS		VIRESCENTS		TOTAL
	Red-stem	Non-red-stem	Red-stem	Non-red-stem	
Observed	208	63	70	28	369
Calculated, 45 percent crossing over	*212*	*65*	*65*	*28*	*370*

$\chi^2 = 0.521$, $P = 0.87$

Here P shows an excellent fit of the observed and calculated. The seedlings in this case were grown later in the season when there was more sunlight.

Progeny of the three F_2's whose segregation appears at the top of table 29, showed in F_3 the distribution with respect to genotypes indicated in table 14.

TABLE 14

GENOTYPES	OBSERVED	CALCULATED, 45 PERCENT CROSSING OVER
V_1V_1AA	3	1.4
V_1V_1Aa	2	2.9
V_1v_1AA	1	2.9
V_1v_1Aa	7	5.8
Total	13	13.0

$\chi^2 = 3.601$, $P = 0.31$

In this progeny test, P shows that there is some agreement between the calculated and observed classes.

Further evidence of the linkage of V_1 and A is found in another series of related families. The families L2281, L2282, L2283 from one ear; L2284, L2285, L2286 from another ear; and both original ears are from an old family of Dr. LINDSTROM's, namely, L882; and family L2303 is the product of a cross in which one individual of L882 was the female parent. In the progenies involved here there is a distinct segregation of the Aa factor pair which is shown by the fact that in these families there appeared plant color types with the A factor as well as those not carrying the A factor. A total of all individuals from heterozygous parents gave a good 3 : 1 ratio with reference to this factor, that is, 232 : 75. If R^rr^g, R^rR^g or R^gr^r had been involved, then a ratio of 15 anthocyanin types to 1 green or non-anthocyanin type would have been expected, as the families were segregating for the Bb factor pair as well (ABR^r = anthocyanin, ABR^g = anthocyanin, AbR^r = anthocyanin, while AbR^g = full green or non-red). That R^g was not involved is also shown by the fact that there appeared no non-red-stemmed seedlings in the colored aleurone classes nor any full green (or non-red) plants in the colored aleurone classes even in the field. From all angles it seems that it is the Aa factor pair which is involved in the linkage shown with the V_1v_1 factor pair.

Table 30 and table 31 give the seedling counts of those progenies segregating for four classes where the coupling and repulsion phases, respectively, are involved.

The crossover value was found to be 45 percent. The value of 45 percent crossing over gives a gametic ratio of about 1.2 to 1, with an F_2 pheno-

typic ratio for coupling, of 11.12 *AB* : 3.4 *Ab* : 3.4 *aB* : 1.44 *ab*, and for repulsion a phenotypic ratio of 10.68 *AB* : 3.84 *Ab* : 3.84 *aB* : 1 *ab*.

A summary of results for the coupling phase is presented in table 15.

TABLE 15

	GREENS		VIRESCENTS		TOTAL
	Red-stem	Non-red-stem	Red-stem	Non-red-stem	
Observed	1722	511	501	220	2954
Calculated	*1697*	*519*	*519*	*220*	*2955*

$\chi^2 = 1.115$ $P = 0.77$

The repulsion phase is summarized in table 16.

TABLE 16

	GREENS		VIRESCENTS		TOTAL
	Red-stem	Non-red-stem	Red-stem	Non-red-stem	
Observed	974	373	352	97	1796
Calculated, 45 percent crossing over	*991*	*356*	*356*	*93*	*1796*

$\chi^2 = 1.322$ $P = 0.73$

In both the coupling and repulsion phases the value of P is above 0.7, which indicates that there is a good agreement between the hypothesis and the facts. The deviations may, therefore, be explained as being due to random sampling.

In some families with small numbers, it was hard to draw an absolute line between the coupling and repulsion phases. Where there was doubt as to which phase a certain progeny belonged, calculations were made to fit the observed to both the coupling and the repulsion phases and the progeny was thrown into the one which gave the closest fit.

In finally testing the relation of V_1v_1 and *Aa* in this series of families their progenies were classified according to the hypothesis of 45 percent crossing over. Only the progenies falling into the four classes of genotypes AAV_1V_1, $AA\ V_1v_1$, $Aa\ V_1V_1$ and $Aa\ V_1v_1$, are used. The $AA\ v_1v_1$, $Aa\ v_1v_1$, and $aa\ v_1v_1$ are virescents and usually die in the field or else seldom produce ears (LINDSTROM 1918). The $aa\ V_1v_1$, and $aa\ V_1V_1$ genotypes are devoid of anthocyanin and only a few were self-pollinated in the field for the reason that they never give the desired four-class segregation.

The results of the progeny test are given in tables 17 and 18.

TABLE 17

Coupling phase (parents 44-6; 2303-5; 39-10; 39-18).

GENOTYPE	EXPECTED RATIO FOR 45 PERCENT CROSSING OVER	OBSERVED	CALCULATED
$AA\ V_1V_1$	1.44	3	3.2
$Aa\ V_1V_1$	2.40	8	5.3
$AA\ V_1v_1$	2.40	5	5.3
$Aa\ V_1v_1$	4.88	9	10.7
Total	11.12	25	24.5

P=0.65

TABLE 18

Repulsion phase (parents 2281-14; 44-3; 38-8).

GENOTYPES	EXPECTED RATIO FOR 45 PERCENT CROSSING OVER	OBSERVED	CALCULATED
V_1V_1AA	1.00	1	1.9
V_1V_1Aa	2.44	4	4.6
V_1v_1AA	2.44	2	4.6
V_1v_1Aa	4.80	13	9.1
Total	10.68	20	20.2

P=0.31

These results of the progeny test fit very well with the hypothesis of 45 percent crossing over, as is shown by P.

To be sure of the placing of Aa and V_1v_1 factor pairs in the same linkage group, a factor or factors will have to be found which lie between the two in order to check the loose linkage which is found here. These results are only approximations of the true linkage, if they are linked, and all that can be said at this time is that the results here indicate 45 percent crossing over between the two factor pairs.

INDEPENDENCE OF V_1v_1 AND P_lp_l FACTOR PAIRS

In order to have a check on the dark-room method used in testing P_l and W, results from some virescent material, tested by the dark-room method, will be given. Crosses were made involving the V_1v_1 and P_lp_l factor pairs. Three ears from one cross, dilute-purple, $P_lp_l\ V_1v_1$, with

dilute-sun-red, p_1p_1 V_1V_1, were obtained in F_3 which segregated in the dark.

The results of these three ears are given in table 19.

TABLE 19

PEDIGREE NUMBER	RED-STEM IN THE DARK		NON-RED-STEM IN THE DARK	
	Green	Virescent	Green	Virescent
373–1	120	48	110	24
373–5	45	20	23	5
373–3	25	8	44	18
Total	190	76	177	47
Calculated, (3 : 1 ratios)	*199*	*67*	*168*	*56*
Deviation	9	9	9	9

$$\frac{\text{Dev.}}{\text{P.E.}}=\frac{9}{4.76}=1.9$$

The deviation divided by the probable error shows independent inheritance of V_1 and P_1, and that growing the plants in the dark-room did not affect the degree of linkage.

LINKAGE OF A FACTOR CAUSING ALBINO SEEDLINGS WITH A FACTOR PAIR CAUSING RED- AND NON-RED-STEM SEEDLINGS

In the spring of 1920 a part of two families which originally came from Dr. W. E. CASTLE, was turned over to the writer. These two families, CASTLE'S Nos. 8 and 224, open-pollinated yellow flint, were sent to CORNELL UNIVERSITY, where they were grown for one year by Doctor LINDSTROM, who self-pollinated many ears. He also continued a part of one (No. 8) for another year; some of this latter material also was turned over to the writer. The material actually turned over included those progenies from number 8 which indicated a linkage of albino seedlings with a red- and a non-red-stem seedling character, and an individual ear, which originally came from No. 224, which showed independent inheritance of these mentioned characters.

The albino character found here seems to be identical with that reported by LINDSTROM. No better specimens of albino seedlings could be found than these, as they are practically pure white.

The material in which W_2 (a duplicate factor for albino seedlings) appears, happens to be very early, and this was a decided disadvantage in making crosses with other types. During the first two summers that the

writer grew this material, the early parts of the seasons were very dry, especially at the time the material was flowering. This was a disadvantage in that the pollen was viable for only a short time. This made even self-fertilizations difficult. Suckers were very few in number and those that were found produced very little pollen. To overcome this difficulty two plantings were made in the summer of 1922, one early and one late. Difficulty in obtaining self-fertilizations was probably also due to a difference between the time of dehiscence of the anthers and the emergence of the silks, a difference which was noted in these families. Pollen sterility is another probable cause, but this phase will have to be left to later investigation. That pollen sterility may have been partly the cause of poor catches is shown by the fact that out of large numbers of self-pollinations made, many never produced a grain, others produced a few kernels, and altogether only a very few ears were obtained which were practically normal.

In some of this material there appears a defective endosperm character which might have some influence on the sterility or the poorness of catches, though this is, as yet only speculation. The major cause of the poorness of this material may be due in general to inbreeding.

The red- and the non-red-stem characters found here have not checked thus far with anything we know in the *A B* P_1 series. The classes of stem seedlings that were described in an earlier section of this paper will apply here. The red- and the non-red-stem classes in this material are very distinct and especially is this true in the white classes. Under some conditions it is very difficult to classify the red- and the non-red-stems within the green-chlorophyll class, and under some conditions it is even difficult among the whites. In totals of red : non-red stems there appeared numbers in the green-stem class out of proportion to a 3 : 1 ratio, but there is scarcely any doubt that this is due to the difficulty in classification within the greens as to stem color, as noted above.

Previously to the 1922 season all the mature plants of CASTLE's No. 8 in the field, were classified as dilute-reds. In the season of 1922, some full-green plants appeared. In the early part of 1923 some non-red-stem seedlings were marked and observed throughout their growing period. No red pigment was found at any stage on these marked plants. The season of 1922 was very favorable for the development of color. In the preceding two years the season was very dry, and therefore very unfavorable for proper development of color, especially for distinguishing weak dilute-sun-reds from full-greens or non-reds. If the green plants that

appeared are true full-greens, then the red and non-red character which is found in these cultures is due to either an *A* factor or the R^g factor.

In table 31 is found the distribution among progeny of all ears which segregated for the four classes, namely, green red-stem, green non-red-stem, white red-stem, and white non-red-stem.

The summary of the results of the coupling phase is given in table 20.

TABLE 20

	GREENS		WHITES		TOTAL
	Red-stem	Non-red-stem	Red-stem	Non-red-stem	
Observed	1329	202	171	402	2104
Calculated, 5 : 1 gametic ratio	*1416*	*161*	*161*	*365*	*2103*
o−c	−87	+41	+10	+37	

The results here point to about 17 percent crossing over, but the deviation of calculated from observed is so great that a good fit would be impossible. This may be due to several things. It is to be noted that within the two non-red-stem classes the deviations are plus, while in the red-stem classes one large deviation is minus. This is due probably to counts made early, before the red had a chance to develop. In a number of these progenies three counts were made of each planting and observations on the difference between these counts, taken two or three days apart, showed that there was a shifting of the non-red-stems to the red class in the second counting, while in the third the counts were practically the same as the second. This shows that if the counts were taken at an early date, an excess of non-red-stems would be obtained. At the best this coupling phase indicates about 17 percent crossing over.

In taking the distribution of stem color within the white class alone, the calculations shown in table 21 were made.

TABLE 21

	RED-STEM	NON-RED-STEM	TOTAL
Observed	171	402	573
Calculated, 17 percent crossing over or 5 : 1 gametic ratio	*175*	*398*	*573*
Deviation	−4	+4	

The deviation here is small enough to say that it is due to random sampling.

One unmistakable case of repulsion was found. The counts in this case were taken directly from Doctor LINDSTROM's records. The results are given in table 22. (The individual ear was L2258-15, and unfortunately was never used as a parent).

TABLE 22

	GREENS		WHITES		TOTAL
	Red-stem	Non-red-stem	Red-stem	Non-red-stem	
Observed	43	22	16	2	83
Calculated, 17 percent crossing over or 5 : 1 gametic ratio	*42*	*20*	*20*	*1*	*83*

$\chi^2=2.0$ $P=0.57$

Here, although the numbers are very small, P gives a very good fit for 17 percent crossing over. There seems to have been no difficulty in classification probably because counts were made late, after pigment had developed well.

The results of the progeny test, which is the most significant test of all, are given in table 23.

TABLE 23

GENOTYPES	EXPECTED GAMETIC RATIO 5:1 OR 17 PERCENT CROSSING OVER	OBSERVED	CALCULATED
$W_2W_2R_1R_1$*	25	6	8.5
$W_2W_2R_1r_1$	10	3	3.4
$W_2w_2R_1R_1$	10	3	3.4
$W_2w_2R_1r_1$	52	21	17.7

$\chi^2=1.501$ $P=0.687$

* R_1 and r_1 = red- and non-red-stem, respectively (arbitrarily used).

From these results W_1 and the red- and the non-red-stem factors are undoubtedly closely linked.

It was stated previously that the factors concerned here in producing red- and non-red-stem seedlings parallel what one would expect if either the A or the r^g factor were concerned. The evidence as to which one, so far as it is available at the present time, is given below.

In a cross, 287 (14) by 305 (5), both of which were dilute-sun-reds, and the F_1's of which were all dilute-sun-reds, there is found some evidence

which points to the r^g factor as being the one concerned in the linkage with W_2. In the F_1's of the cross five self-pollinations were made and all ears grown in the greenhouse. Counts were obtained on only two, the others being completely destroyed by mice. In one of these two, a 3 : 1 ratio of red-stem : non-red-stem was found, but in the other a 9 : 7 ratio of red- : non-red-stem was exhibited. The counts are given in table 24.

TABLE 24

849-3, F_2 of 287-14×305-5.

	RED-STEM	NON-RED-STEM
Observed	145	106
Calculated, 9 : 7 ratio	*141*	*110*
Deviation	4	4

If the factor pair for red and non-red stems concerned here was the $r^r r^g$, then from a cross $AAr^r r^g$ (287-14) with $Aar^r r^r$ we would have in F_1 four genotypes, namely, $AAr^r r^r$, $Aar^r r^r$, $AAr^r r^g$, and $Aar^r r^g$. The latter genotype would give, when selfed, a segregation of 9 red-stem : 7 non-red-stem. Now, it is known that in the 305 family the factor concerned in producing red- and non-red-stems is the A factor, as this family is derived from old stocks of Doctor LINDSTROM'S. But, in the 287 family, where the linkage of W_2 and a factor for red and non-red stems is found, the factor producing this red and non-red is not known. It is undoubtedly, as has been said, due to either A or r^g and the argument is given below. If both had been heterozygous for the same factor only 3 : 1 ratios would be obtained. But we have one here that gives us a distinct 9 : 7 ratio. We might under normal conditions get a deviation in a monohybrid which would give us a 9 : 7 where a 3 : 1 is expected, but this occurs very seldom. The result of this 9 : 7 progeny is fitted to a 3 : 1 ratio in table 25.

TABLE 25

	RED-STEM	NON-RED-STEM
Observed	145	105
Calculated, 3 : 1	*188*	*62*
Deviation	43	43

Deviation ÷ probable error = 9.35

The odds are so great against the deviations being the result of random sampling that the former calculations will have to suffice. Had the red- and non-red-stem factor in the 287 family been the *A* factor, 9 : 7 ratios could never have been obtained in the cross stated above. The *A* which came from the 305 family, in combination with the r^g from the 287 family would be sufficient to account for the result obtained.

Accordingly, this bit of evidence points to the *R* instead of the *A* locus as being the one linked with W_2. This checks with some of the unpublished results obtained by Doctor LINDSTROM where a W_2 is concerned. He gets 16 percent crossing over. There is about 17 percent crossing over here, which checks very closely with his results.

Further evidence which points to R^g or r^g as being the factor concerned here in linkage with W_2 comes from a cross of 377-1 $W_1W_1\ W_2w_2$ ($AA\ r^rr^g$?) dilute-sun-red, with 325-22 $W_1W_1\ W_2w_2$ ($aa\ r^rr^r$) full-green or non-red. In the F_1 generation, all the mature plants were dilute-sun-reds, showing that the *A* factor was present. If 377-1 had contained *AA* we should have expected this to be the case and then in F_2 all the ears would have segregated, upon planting, into 3 reds to 1 non-red. If 377-1 had contained *Aa*, then we would have expected in F_1 a 1 : 1 ratio of red : non-red. The facts of the case are that in F_1 no segregation took place and in F_2 all progenies segregated for reds and non-reds. The segregation was of two kinds, one giving a 3 : 1 ratio, and the other kind an approximation to a 9 : 7 ratio. If the constitution of 377-1 was $AA\ R^rR^g$ and 325-22, $aa\ R^rR^r$, the results obtained are what one would expect. In such a case there would be two kinds of F_1's, namely, $Aa\ R^rR^r$ and $Aa\ R^rR^g$. In F_2 the former would give a 3 : 1 ratio of red- : non-red-stems and the latter a 9 : 7 ratio of the same characters.

The results are shown in table 26.

TABLE 26

PEDIGREE NUMBER	RED-STEM	NON-RED-STEM	APPROXIMATE RATIO
352 (1)	10		9 : 7
(3)	85	77	9 : 7
(4)	142	66	3 : 1
(5)	18	16	9 : 7
(6)	13	17	9 : 7
(7)	22	12	9 : 7
(8)	14	56	3 : 1
(9)	9	9	9 : 7

Most of these eight progenies have small numbers, and it is hard to identify some of them with 3 : 1 or a 9 : 7 ratio; among the others there is no doubt as to some being 3 : 1 and others 9 : 7.

A significant fact is brought out by the following relation. In a cross of the 377 family wherein the linkage of W_2 and a factor for red- and non-red-stem seedlings is concerned (it also gave a 3 : 1 ratio of red- : non-red-stem seedlings) with another family (325) where the A factor is known to be concerned in producing 3 red- : 1 non-red-stem seedlings, there are obtained 9 red- : 7 non-red-stem seedlings. Accordingly, if factors which were producing 3 : 1 ratios in their respective lines gave on combination 9 : 7 ratios, the only logical conclusion is that these factors are different. It follows, then, that if one is known to be A the other is presumably r^g, since only these two factors are at present known to be responsible for red- and non-red-stem plants.

This, then, seems to show that the R^g is present here and responsible for the red- and non-red-stem character concerned in the linkage with W_2w_2. This is contradictory to the evidence pointed out where CASTLE'S other line is considered. This other line, CASTLE'S No. 224, although there is no direct heritable connection of it with No. 8 (the line considered above in the linkage of W_2 and red and non-red stem) was supposed to contain the same W, but from this it seems to be certain that these two factors for albino seedlings are different.

A brief account will be given of CASTLE'S No. 224 which shows an independence of a W with a factor causing red- and non-red-stem seedlings.

The segregation of the progeny and the results are given in table 33. The value of P is 0.19, which is not a very good fit, although within the white class the deviation divided by the probable error equals 1.5. Judging from the data of the white class, it is reasonable to say that the two factors concerned here are independent of each other.

To test the presence and absence of A, crosses were made between one family, 290 (from 35-1), which was composed of all full-greens, with brown plants, aBP_l. With such a cross if A were present we would expect full-anthocyanin plants in the next generation (EMERSON 1921), but if A were absent only plants with the absence of any red pigment would appear. Crosses, 290-31 E gr.[3] with 372-10 brown, and 290-32 E gr. with 372-10 brown, were made, and their progeny grown in the greenhouse the following winter. In every case the entire progeny had red stems while not a red-stem plant appeared in the selfed plants of No. 290. Family 290 was

[3] E gr. = full-green plant.

selected for the test of this stem character, as it was composed of only full-greens or non-red plants.

Also the crosses, 290-22 E gr. with 384-12 E gr.; 290-25 E gr. with 285-4 E gr.; 290-48 E gr. with 303-14 E gr.; gave only red-stem progeny.

A cross 290-45 E gr. with L4247-1 D gr.[4] *R* tester, gave only colorless kernels, showing that the *r* was present here and not *R*.

From the above data of progenies of CASTLE'S 224 it seems certain, then, that the factor concerned in causing red- and non-red-stem seedlings here is the r^g factor. At least it parallels the results that one would expect if r^g were present, and according to EMERSON (1921) the test with browns and greens is the logical test for r^g. So we can conclude that the *W* concerned here is independent of *R*.

From these data it is not absolutely safe to draw definite conclusions as to the factors concerned for albinos and red- and non-red-stem seedlings reported above. The evidence, however, seems to point to one factor for red- and non-red-stem seedlings, the r^g, and two factors for albino seedlings. The albino factor in CASTLE'S No. 8 (W_2) is definitely linked to a factor for red- and non-red-stem seedlings and evidence is brought forward, to show that this factor is r^g. Then, if this is true, the albino factor in CASTLE'S 224 is an entirely different factor from W_2, as it is independent of the r^g.

SUMMARY

The independent relationship of W_1 and *A* is reported. The possibility of a very loose linkage relationship between these two factors is also presented.

By means of a dark-room test the factors P_l for plant color and W_1 for albino seedlings were found to be linked. The crossover value approximated 25 percent.

A loose linkage was found between *Y* and W_1 with a crossover value of 42 percent.

The arrangement of the three above linked factors is Y-P_l-W_1. The chromosome map of this group is shown, including the S_m factor for silk color.

The *Aa* factor pair for aleurone and plant color was found to be loosely linked with the V_1v_1 factor pair for green-virescent seedlings. The amount of crossing over was 45 percent.

By means of the dark-room test an independent relationship was found between P_l and V_1.

[4] D gr. = dilute-sun-red.

A linkage is reported which involves a factor causing albino seedlings, W_2, with a factor pair causing red- and non-red-stem seedlings (r^g?). The crossover value was found to be about 17 percent.

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APPENDIX—Tables 27 to 33

TABLE 27

F_2 dihybrid segregation of the cross L3360-28×L3349-7 exhibiting 42 percent crossing over between Y and W_1.

PEDIGREE	YELLOW SEED		WHITE SEED		SEED PLANTED		TOTAL CHLOROPHYLL	
Numbers	Green	White	Green	White	Yellow	White	Green	White
L4144–2	110	35	30	19	150	50	140	54
L4144–13	64	19	21	6	115	41	85	25
L4144–14	108	38	38	12	150	50	146	50
L4144–16	118	27	34	14	150	50	152	41
L4144–20	119	32	32	15	150	50	151	47
L4144–21	84	24	30	24	150	50	114	48
L4144–23	124	34	38	26	158	65	162	60
Total	727	209	223	116	1023	356	950	325

TABLE 28

Segregation of the progenies from yellow seed of L2205-10, W_1w_1Yy.

PEDIGREE NUMBER	YELLOW SEED		WHITE SEED		SEED PLANTED		TOTAL CHLOROPHYLL	
	Green	White	Green	White	Yellow	White	Green	White
L3348–2	103	34	0	0	56	0	103	34
L3348–3	57	0	16	0	63	25	73	0
L3348–4	79	17	15	6	116	29	94	23
L3348–4	30	10	13	6	51	21	43	16*
L3348–5	17	0	8	0	25	10	25	0
L3348–6	25	0	10	0	27	12	35	0
L3348–7	56	0	0	0	63	0	56	0
L3348–8	78	27	19	16	124	38	97	43*
L3348–10	80	20	27	14	128	61	107	34*
L3348–12	50	14	0	0	100	0	50	14
L3348–14	145	61	78	12	220	99	223	73*
L3348–16	101	34	0	0	152	0	101	34
L3348–20	57	9	0	0	74	0	57	9
L3348–21	105	29	35	14	140	55	140	43
L3348–22	71	32	0	0	106	0	71	32
L3348–24	33	9	15	3	98	24	48	12*
L3348–26	16	0	2	0	17	3	18	0
L3348–27	80	0	0	0	88	0	80	0
L3348–28	152	62	49	16	247	81	201	78*
L3348–49	29	9	0	0	40	0	29	9
L3348–31	86	21	0	0	114	0	86	21
L3348–32	159	58	59	13	230	80	218	71*
L2205–10	165	46	59	10	245	84	224	56*

*Dr. LINDSTROM's counts included.

TABLE 29

F_2 and F_3 segregation of a cross whose F_1 was of the arrangement $\widehat{V_1 a}\ \widehat{v_1 A}$ and which shows 45 percent crossing over between V_1 and a and v_1 and A.

PARENT NUMBER	PEDIGREE NUMBER	GREENS		VIRESCENTS	
		Red-stem	Non-red-stem	Red-stem	Non-red-stem
	54–3	46	16	12	3
	54–4	50	8	12	6
	54–5	68	17	12	3
	Total F_2	164	41	36	12
54–4	371–3	83	27	39	11
	371–4	0	19	0	0
	371–5	12	0	0	0
	371–7	88	30	19	9
	371–9	48	16	20	5
	371–10	143	36	65	11
	371–12	21	7	0	0
	371–13	125	36	31	17
	371–14	116	38	48	6
	371–15	133	28	39	9
54–3	369–5	0	128	0	35
	369–8	23	0	0	0
	369–12	0	19	0	12
	369–14	23	0	0	0
54–5	372–1	9	0	6	0
	372–2	28	5	0	0
	372–5	0	20	0	9
	372–9	0	35	0	0
	372–11	0	32	0	0
	372–16	0	25	0	4

TABLE 30

Coupling-phase segregation of progenies in F_3 and later generations from parents heterozygous for V_1v_1 and Aa factor pairs and which show 45 percent crossing over.

PEDIGREE NUMBER	GREENS		VIRESCENTS	
	Red-stem	Non-red-stem	Red-stem	Non-red-stem
L2281–5	27	4	7	3
L2281–9	38	5	9	4
L2281–13	28	8	7	5
L2281–11	98	38	31	13
L2281–26	25	9	6	5
44–6	64	20	19	11
315–4	121	34	32	14
317–3	67	19	19	7
317–8	218	80	68	21
L2284–22	46	5	10	1
L2286–16	32	8	7	3
L2286–18	22	6	11	4
L2286–19	22	6	8	6
L2284–12	29	11	7	5
311–6	118	23	22	19
311–8	47	13	12	6
L2303–5	38	16	11	6
L2303–8	41	8	8	4
L2303–12	53	17	16	7
L2303–17	34	8	15	3
39–18	35	28	10	9
39–10	33	8	6	2
299–1	97	24	38	13
299–19	129	38	45	13
300–27	51	8	10	6
303–16	126	48	41	19
304–4	83	19	26	11
Total	1722	511	501	220

TABLE 31

Repulsion-phase segregation of progenies in F_3 and later generations involving V_1 and A factor pairs with 45 percent crossing over.

PEDIGREE NUMBER	GREEN		VIRESCENT	
	Red-stem	Non-red-stem	Red-stem	Non-red-stem
L2281–14	25	8	12	2
L2282–3	16	3	10	3
44–3	41	6	8	0
44–7	20	9	8	2
45–11	39	14	17	4
315–6	12	4	5	2
317–2	85	26	29	8
L2284–4	13	8	10	3
L2284–6	31	9	9	0
L2284–8	22	10	12	3
L2284–23	30	9	6	2
L2285–2	30	13	9	3
L2286–22	23	17	4	1
42–8	8	6	3	1
311–2	56	22	16	4
311–17	34	17	22	5
312–10	132	34	38	9
312–12	72	20	10	3
38–8	58	33	24	5
38–11	22	8	4	1
299–8	111	37	49	16
299–9	94	47	39	16
300–20	6	9	7	1
316–1	4	4	1	3
Total	984	373	352	97

TABLE 32

Segregation of the progenies whose parents were heterozygous for W_2 and a red- and a non-red-stem character. (From CASTLE'S *No. 8).*

PEDIGREE		GREEN		WHITE	
Parents	Progeny	Red-stem	Non-red-stem	Red-stem	Non-red-stem
CASTLE No. 8	L2258–5	70	0	22	0
	L2258–7	0	54	0	25
	L2258–15	43	22	16	2
	L2258–16	58	0	18	0
	L2258–32	83	16	11	24
	L2258–38	7	13	0	0
	L2258–49	16	16	0	0
	L2258–54	18	11	0	0
L2258–32	L3351–1	54	3	0	10
	L3351–3	10	0	2	0
	L3351–4	0	52	0	10
	L2251–7	28	12	0	0
	L2251–13	53	10	10	12
	34–1	2	0	0	1
	34–4	61	8	9	21
	34–9	32	2	7	12
	34–12	33	0	0	0
	34–10	2	0	0	0
	34–14	16	2	4	9
	34–13	39	8	3	4
34–9	287–1	129	12	15	45
	287–4	87	20	22	60
	287–9	126	19	18	48
	287–10	133	14	12	36
	287–12	81	15	12	31
	287–13	61	5	5	13
34–13	288–1	113	17	7	28
	288–2	80	18	19	20
	288–3	24	7	0	0
	288–5	72	15	8	15
	288–10	31	0	0	0
	288–13	12	2	0	1
	288–15	17	3	1	1
	288–16	21	0	3	0
	288–17	16	0	0	0
	288–18	17	10	3	0
34–14	289–3	0	0	0	0
	289–4	29	0	6	0
	289–6	30	0	0	0
	289–7	41	25	0	0
L3351–13	377–3	45	0	0	0
	377–7	56	10	8	9
	377–8	22	3	0	2

TABLE 33

Segregation in F_3 and later generations where a W factor and a factor for red- and non-red-stem seedlings are independent. Derived from CASTLE'S *number 224.*

PEDIGREE		GREEN		WHITE	
Parents	Progeny	Red-stem	Non-red-stem	Red-stem	Non-red-stem
CASTLE 224	L2215–2	83	35	21	10
	L2215–22	82	32	20	7
	L2215–29	13	5	6	1*
	L2215–29	11	4	5	4
L2215–29	35–1	0	73	0	14
	35–9	35	8	0	0
	35–16	25	10	8	3
Total		214	86	60	25

*Dr. LINDSTROM'S counts.

STUDIES ON REPRODUCTION IN *AMOEBA PROTEUS*[1]

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Received November 17, 1923

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INTRODUCTION

Fundamental significance in so many different directions is commonly attributed to Amoeba that detailed knowledge of its genetic phenomena is desirable. The following paper, dealing with Amoebae of the "*proteus*" types, contributes observations on the constancy of characteristics in diverse types when cultivated under similar conditions; on the rate and rhythmic character of vegetative reproduction in different strains; on the effects of diverse cultural conditions upon reproduction; and on the origin and methods of reproduction of polynucleate forms.

MATERIAL AND METHODS OF CULTURE

The Amoebae employed were partly obtained from the laboratory cultures of Dr. J. G. EDWARDS; partly from a pond in the Botanical Garden of the JOHNS HOPKINS UNIVERSITY. Pedigree cultures were followed

[1] From the Zoölogical Laboratory of THE JOHNS HOPKINS UNIVERSITY.

by isolating single individuals in the concavities of hollow-ground slides, each concavity containing 0.2 cc of culture fluid. The slides were kept in moist chambers and examined daily as to fission. Fresh culture fluid was supplied at different intervals in different experiments, as will be set forth. Pedigree records were kept by giving a designation to each individual, on the following system: The parent individual is designated N. 1. (N being the number of the line). The products of the first fission are designated N.2 and N.3. The offspring of N.2 are N.4 and N.5; those of N.3 are N.6 and N.7. This process is continued for ten generations, the individuals of which have a range of numbers from N.1024 to N.2047. The designation of the individuals which are to be continued in the pedigree is then reduced to N. Ia1, N. Ib1, etc. The formula used above is employed again and continued for another ten generations. The designation of the individuals of the twentieth generation is reduced to N. IIa1, N. IIb1, etc. The pedigree can be continued indefinitely, each member of the pedigree having as its identification mark the number of the family, the number of the generation reached, and the line of descent. All pipettes, slides, bottles and dishes were washed in boiling water after havng been used once, to eliminate any possibilities of contamination from unknown sources.

A hay-infusion infusoria culture medium was employed, as by STOLC (1905) and SCHAEFFER (1917). It is prepared in the following manner: Stalks of dry timothy hay are cut into half-inch strips and autoclaved. Two hundred cubic centimeters of distilled water are placed in a finger bowl (diameter 4 inches) and on the surface of the water is spread one gram of the sterilized hay. This is left exposed to the air. In a few days the hay begins to decay, the surface of the liquid serving as a nutrient medium for a variety of bacteria, yeasts and moulds. At ten-day intervals, the evaporated liquid is replaced by equal amounts of distilled water (50-75 cc). Fifteen days after the preparation of the culture, it is inoculated with 50 cc of a dilute hay-infusion fluid which contains numerous chilomonas and smaller infusoria, but no rotifers. The infusoria feed on the micro-organisms, and multiply rapidly. Fifteen days after inoculation, the medium is filtered through a coarse filter paper into one-half pint milk bottles. It is now reâdy to serve as a culture fluid for Amoebae. These cultures were renewed at ten-day intervals.

These studies were undertaken at the suggestion of Professor H. S. JENNINGS in November 1921, and were attacked by the method of isolation pedigree cultures used by the investigators on inheritance in unicellular organisms at THE JOHNS HOPKINS Zoölogical Laboratory. The writer is indebted to Professor H. S. JENNINGS for his valuable counsel and aid.

CONSTANCY OF THE CHARACTERISTICS OF DIVERSE TYPES

The Amoeba figured in text-books of elementary biology was discovered by RÖSEL in 1755. He named the specimen that he had found "der kleine Proteus." PALLAS in 1766 redescribed and renamed this organism, "*Volvox proteus*." EHRENBERG found the same form in 1830, but described it under a new name, "*Amoeba diffluens*." One year later he described a large Amoeba of the same type, and differentiated it from his first find by giving it a new specific name, "*Amoeba princeps*." EHRENBERG'S Amoebae became confused by later investigators. A number of observers described the same organism, or slight variations of it, under new specific names, e.g., *Amoeba ramosa* Fromentel; *Amoeba communis* Duncan, and *Amoeba villosa* Wallich. Thinking that the environment had a great deal to do with the size and the general appearance of the Amoebae, also seeking to eliminate this confusion of description and nomenclature, LEIDY first grouped all these forms under the name *Amoeba chaos*, but finally, in his survey of 1879, decided on the name *Amoeba proteus*. This simplified matters a great deal, but several students of Amoebae protested at this wholesale grouping of all the variations under one name.

With each protest came new specific names. PENARD (1902) divided the group into three species, viz., *Amoeba proteus*, *Amoeba nobilis* and *Amoeba nitida*, using nuclear differences as the basis of his classification. CASH and HOPKINSON (1905) found that twenty-three names had been applied to the *Amoeba proteus* described in LEIDY'S work.

SCHAEFFER (1916) found that the morphological differences in various forms of *Amoeba proteus* were accompanied by corresponding differences in behavior to many food substances. He consequently broke the group up into (a) the granular type and (b) the raptorial type, and divided *Amoeba proteus* (Leidy) into three species, as follows:

(a) Granular type.

1. *Amoeba proteus* Pallas (Leidy). About 600 microns; longitudinal ridges on pseudopods; discoidal nucleus which may be folded in old individuals.

2. *Amoeba discoides* sp. nov. About 400 microns; no ridges; discoidal nucleus; numerous, small crystals.

(b) Raptorial type.

3. *Amoeba dubia*, sp. nov. About 400 microns; no ridges; few, large crystals; ovoidal nucleus.

CARTER (1919) accepted SCHAEFFER'S separation of *Amoeba proteus* into the granular and raptorial types, but protested against the addition

of new specific names to the already overburdened nomenclature list of the organism. She put forward the hypothesis that all these Amoebae belong to a single species and submitted the following classification for use until the discovery of the complete life-cycle of *Amoeba proteus*.

(a) Granular Amoebae.

1. *Amoeba proteus* Pallas, var. *granulosa*.
   Amoeba princeps Ehrenberg } *Amoeba proteus* Y.
2. *Amoeba nobilis* Penard, multinucleate — *Amoeba proteus* Z.

(b) Raptorial Amoebae.

3. *Amoeba proteus* Pallas. — *Amoeba proteus* X.

The opinion has at times been expressed that the many diverse types of Amoeba are constitutionally the same, the diversities being merely the result of diverse environment. Others hold that the diverse forms are permanently differentiated, so that they may properly be characterized as different species.

SCHAEFFER (1917) pedigreed each of his three types under controlled conditions and found that the morphological as well as the physiological characteristics of each type remained constant for 15 generations. Previously, STOLC (1906), in a pedigree study of a single line of a granular Amoeba, found that the characteristics of the organism did not change during 292 generations, more than 1 year and 8 months, in isolation culture. CARTER (1919) found a constancy of characteristics existing in a raptorial Amoeba, multiplying by fission for more than 6 months.

To further test the constancy of the differences existing between these two types, seven granular Amoebae (each with longitudinal ridges on the pseudopods), and seven raptorial Amoebae, were isolated by the writer for pedigree study. All the Amoebae were transferred daily to clean slides containing fresh culture fluid. One line of each type was pedigreed for 40 generations, the other lines were studied for shorter periods.

It was found that the marked differences that existed between the two types remained constant, as SCHAEFFER had previously demonstrated. Slight variations in the cytoplasmic and nuclear characteristics in the granular Amoebae arose during the course of the pedigree. The individuals that did not divide for long periods became more densely granular and much larger than the parent form, while those that divided rapidly were, as a rule, less granular and smaller than the parent. The larger individuals were occasionally binucleate and even polynucleate. The nuclei of others of the larger Amoebae were oftentimes bent or wrinkled. But all the modifications were, without exception, of a temporary nature. The

different forms were interchangeable; one could pass into the other, depending upon the condition of the organism and the nature of the culture medium. The raptorial Amoebae showed no apparent signs of divergence from the parent form, throughout the pedigree.

A consideration of table 1 shows that the total number of recorded divisions in all the pedigree lines of the raptorial Amoebae was 626 and the average interval between two successive divisions was 1.6 days. The total number of recorded divisions of the granular Amoebae was 846 and the average interval between successive divisions was 2.5 days. In addition to this difference in the division rate, there was a significant differ-

TABLE 1

Data on the division rate of all the individuals in 7 lines of granular Amoebae and 7 lines of raptorial Amoebae. The different lines were cultivated simultaneously from Feb. 13, 1922 to June 14, 1922. Each individual was transferred daily to fresh culture fluid.

NUMBER OF DAYS BETWEEN DIVISIONS	THE TOTAL NUMBER OF INDIVIDUALS THAT DIVIDED, IN ALL LINES OF	
	Granular Amoebae	Raptorial Amoebae
1	343	377
2	248	187
3	111	37
4	47	6
5	31	5
6	19	4
7	6	4
8	13	5
9	5	...
10	7	1
11		...
12	6	...
13	1	...
14	1	...
16	2	...
17	1	...
18	1	...
19	1	...
Average interval between divisions in days	2.5	1.6

ence between the two types in the length of the period of inactivity (interval during which there is no division). The maximum period of inactivity of the raptorial Amoebae was 10 days, while the granular Amoebae went without dividing for periods of 18 and 19 days (table 1). The raptorial Amoebae differed in still another respect from the granular Amoebae. While a long period of inactivity proved fatal to the former, it had no such

effect on the latter. The net result was a higher death rate among the raptorial forms.

Another difference between the two types was observed when the intervals between successive divisions were averaged for five-day periods (figure 1). This method of presenting results was used by WOODRUFF and BAITSELL (1911) in their studies on the division rates in Paramecium. The graph shows that during the first half of the pedigree the granular

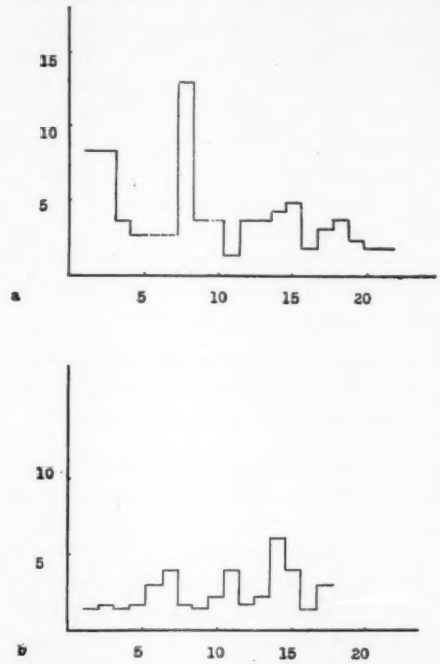

FIGURE 1.—Graphs for the division rates of the individuals in a line of granular Amoebae (a), and a line of raptorial Amoebae (b). The vertical scale shows the average number of days between the divisions of the individuals that divided within each 5-day period; the horizontal scale shows the number of successive 5-day periods. The dotted lines represent periods during which no division occurred. a, graph for the granular Amoebae; b, graph for the raptorial Amoebae.

Amoebae divided very slowly, while the raptorial Amoebae divided daily. The latter divided about four times as rapidly as the granular type. In the last half of the pedigree, the granular Amoebae showed a decided increase in the division rate, while the raptorial Amoebae showed a slight

decrease. Thus the division rate of the two types at the end of the pedigree was about the same. The death of all the raptorial Amoebae prevented a further comparison of the two lines. These results are indicative of an increase in the vitality (fission rate) of the granular Amoebae and a decrease in the vitality of the raptorial Amoebae when both are kept under the same culture conditions.

A further examination of figure 1 shows an interesting rhythmic character in the division rate of each type. It shows that there is an alternation of periods of high and low reproductive activity, which appeared to be of the same nature for both types; also that the value of the several maxima and minima varied, and the interval between two successive maximum division rates was about 25 days. BOTSFORD (1922) found similar rhythms in the reproduction of *Amoeba bigemma*. The exact nature and the importance of these rhythms in the life cycle of the Amoebae has not yet been determined. It seems probable that cytological study will show that the rhythms result from processes similar to endomixis in Paramecium.

My results from pedigree cultures thus confirm those of SCHAEFFER in showing that the differences between the granular and the raptorial forms are constant for long series of generations under identical conditions.

INFLUENCE OF DIVERSITIES IN ENVIRONMENTAL CONDITIONS ON THE RATE OF REPRODUCTION AND THE RHYTHMIC CHARACTER OF THE DIVISIONS IN *Amoeba proteus*

Several investigators have presented evidence that the excretion products of an organism have a profound effect on its growth. WOODRUFF (1911, 1913) shows that the excretion products of Paramecia apparently lower the division rate of this protozoan. ROBERTSON (1921, 1922), on the other hand, maintains that the excretion products of the infusoria have no effect on their division rate. It appears of interest to investigate, not only the effect of excretion products and other changes in the culture medium on the division rate in *Amoeba proteus*, but also the influence of such changes on the rhythmic character of the divisions. WOODRUFF (1917) found that normal changes in the environment of Paramecia have no effect on the length or the character of the rhythm in the divisions of these organisms.

The organisms used in the present investigation were isolated from cultures of Amoebae which had been grown under pure-line conditions for 9 months. The Amoebae were placed in concavities of hollow-ground slides, each concavity containing 0.3 cc of a hay-infusion infusorian culture prepared as described above. Diversities in the environmental conditions

were produced by keeping the Amoebae isolated in the same culture fluid for varying lengths of time. The series used were as follows:

A. Culture medium changed daily. The Amoebae were transferred every 24 hours to clean slides containing fresh culture fluid. (The fresh culture fluid was obtained from a stock medium which was prepared anew every 10 days).

B. Culture medium changed every 2 days.

C. Culture medium changed every 3 days.

D. Culture medium changed every 5 days.

E. Culture medium changed every 10 days. In this group, water which evaporated was replaced at the fifth day by the addition of distilled water.

An examination of the mortality rates (number disintegrated divided by total number isolated) in all the lines of each series showed that certain changes had occurred in the various culture media. In general, the data indicate that within the limits tested (1 to 10 days) the longer the period that an Amoeba is kept in the same culture fluid, the more detrimental the effect on the vitality of the isolated organism (see table 2).

TABLE 2

The relation between the length of time that an Amoeba is kept in the same culture fluid and the mortality rate. The data include individuals of all lines during the entire period of the experiment.

INTERVALS IN DAYS BETWEEN TRANSFERS	TOTAL NUMBER OF INDIVIDUALS ISOLATED	NUMBER OF INDIVIDUALS THAT DISINTEGRATED	MORTALITY RATE IN PERCENT
1	353	21	5.95
2	752	38	5.05
3	591	62	10.51
5	449	55	12.25
10	409	87	21.27

With relation to the rate of multiplication, the five sets of experiments above described were conducted simultaneously for five months, from October 6, 1922, to March 6, 1923. These allowed comparisons to be made of the effects of the same and of different cultural conditions, on different lines, some belonging to the same races, some to diverse races. The results of the various comparisons are as follows:

(1) Effects of diverse cultural conditions on lines belonging to the same race are shown in table 3, in which are summarized the results of keeping under different cultural conditions a set of lines, all isolated from the fifth generation of a single line in series B. The table is to be read as

follows: In the lines that were changed every day, 43 divisions occurred at one-day intervals, 56 at 2-day intervals, and so on; in those changed at 2-day intervals, 58 divided at 1-day intervals, etc., etc. The data given in table 3 as to the intervals between the divisions,—particularly the averages—indicate that there is a progressive decrease in vitality as

TABLE 3

The effect of different environmental conditions on the division rates of individuals of various lines of the same race of Amoeba proteus. Each line is designated by a capital letter that represents the environmental condition to which it is being subjected, and a subscript which indicates the source from which the parent form was isolated.

INTERVAL IN DAYS BETWEEN SUCCESSIVE DIVISIONS	NUMBER OF AMOEBAE THAT DIVIDED, WHEN THE CULTURE MEDIUM WAS CHANGED EVERY			
	Day, in line A_{19}	2 days, in line B_{19}	5 days, in line D_{19}	10 days, in line E_{19}
1	43	58	26	27
2	56	92	46	28
3	25	32	17	16
4	17	14	8	6
5	6	15	5	5
6	8	9	5	4
7	2	10	2	2
8	2	7	2	3
9	..	6	..	1
10	2	2	2	1
11	4	8	1	..
12	1	4	4	1
13	..	2	1	1
14	5	5	1	..
15	3	..	..	1
16	..	2	..	1
17	1	..	..	
18	..	2	..	..
19	..	..	1	1
20	..	..	..	2
24	..	..	..	1
25	..	..	1	..
30	..	..	1	..
32	..	..	1	..
39	..	..	..	1
Average interval between divisions	3.52	3.90	4.01	4.42

the period between transfers to fresh culture fluid is made longer. The graphs in figure 2 show that in all the lines there is a similarity in the succession of high and low periods of reproductive activity, though there is some irregularity in the lengths of the rhythmic periods.

(2) When for the experiments on the effects of changing the animals at different intervals we select lines derived from different original cultures, (and thus from diverse original progenitors) the results are those shown in table 4. The results are more variable than when lines all derived from

TABLE 4

The effect of different environmental conditions on the division rates of lines of different races of Amoeba proteus. Each line is designated by a letter representing the condition to which it is being subjected, and a subscript which indicates the source from which the parent form was isolated.

INTERVAL IN DAYS BETWEEN SUCCESSIVE DIVISIONS	NUMBER OF INDIVIDUALS THAT DIVIDED, WHEN THE CULTURE MEDIUM WAS CHANGED EVERY			
	1 day, in line A_1	2 days, in line B_9	5 days, in line D_{28}	10 days, in line E_4
1	52	18	21	6
2	47	23	22	34
3	15	7	14	11
4	7	16	13	9
5	3	2	12	12
6	8	4	10	14
7	4	1	2	5
8	1	1	3	10
9	5	..	5	4
10	4	..	3	2
11	3	..	2	3
12	1	..	3	2
13	3	..	3	5
14	3	..	1	2
15	..	1	1	3
16	..	1	1	1
17	1	..	1	1
18	..	1	2	..
20	..	..	..	1
21	..	..	1	..
26	..	1	..	..
Average interval in days between divisions	3.42	3.92	5.06	5.16

the same original parent are employed, but they show on the whole the same decrease in reproductive vigor as the animals that are kept longer in the same culture fluid. Graphs of the progress of these experiments show the same rhythms of high and low reproductive rates, with the same irregularity in the length of the rhythms, that are exhibited in figure 2.

(3) Difference in division rate in the progeny of two sister cells. In a number of cases two sister Amoebae were cultivated separately, but

under identical conditions. Some of these twin cultures were changed every 2 days, others every 3, 5 or 10 days. In the B series of experiments (changed every 2 days), two sister cells were used that were derived from a parent Amoeba having a bent nucleus. At its division one of the two

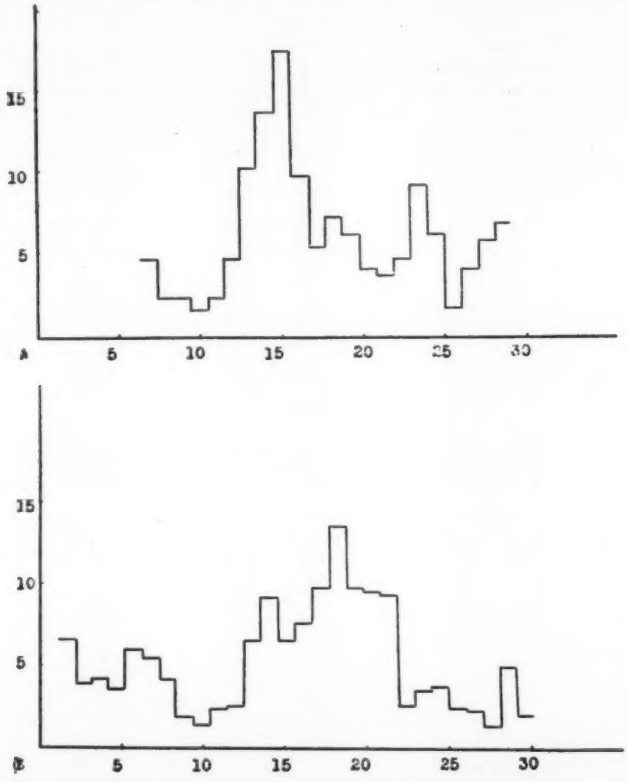

FIGURE 2.—Graphs for the divisions of 4 lines of Amoebae belonging to the same race, but kept under different environmental conditions. The vertical scale shows the average number of days between the divisions of the individuals that divided within each 5-day period; the horizontal scale shows the number of 5-day periods. A, culture medium changed daily; B, medium changed every 2 days; D, medium changed every 5 days; E, medium changed every 10 days.

Amoebae produced had a smooth nucleus, the other a bent nucleus. The former gave rise to the line B2 the latter to the line B2b; the two were cultured under the same conditions. The division rate of the two lines thus derived differed greatly. The line B2b derived from the parent with

bent nuclei multiplies much more slowly (table 5). In line B2 the individuals were larger and had smooth nuclei; in line B2b most of the individuals had bent nuclei. Graphs of the reproduction in the two lines show the usual rhythms.

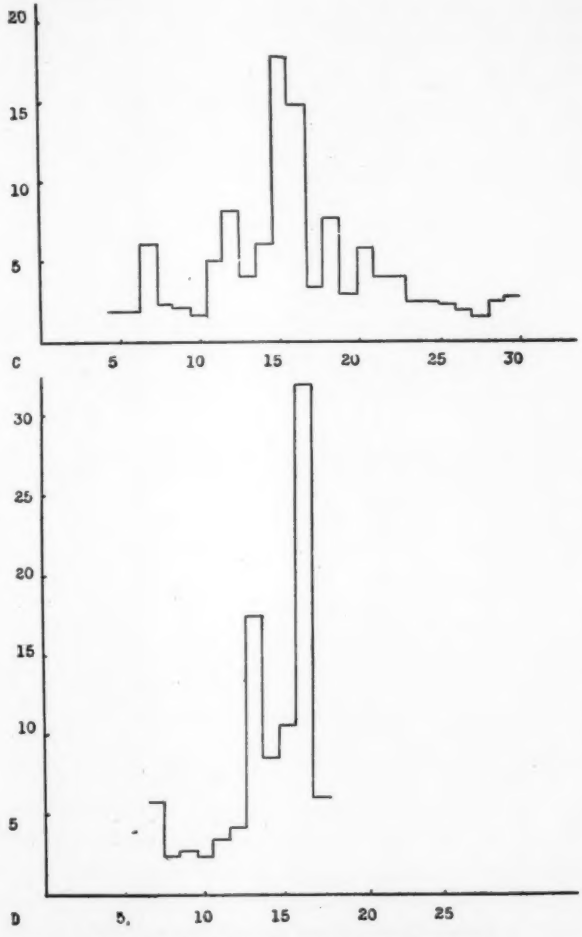

FIGURE 2, C and D. Legend on preceding page.

Three other cases in which sister lines were kept under identical conditions are shown in table 6. The average rate of fission differed somewhat

in the case of the sister lines, C and D, but were identical in the two lines of E. In all these cases the reproduction showed rhythmic alternations of high and low reproductive rates, as seen in the graphs of figure 2.

TABLE 5

The variation in the division rates of the individuals in two lines of the same race of Amoeba proteus when kept under the same environmental conditions. The lines differed in the size and shape of the nucleus. The Amoebae of line B2b invariably had bent nuclei, while those of line B2 usually had smooth nuclei; both were derived from a parent form that had contained a bent nucleus.

NUMBER OF DAYS BETWEEN SUCCESSIVE DIVISIONS	NUMBER OF INDIVIDUALS THAT DIVIDED, WHEN THE CULTURE MEDIUM WAS CHANGED EVERY TWO DAYS	
	In line B2	In line B2b
1	23	4
2	46	7
3	18	8
4	15	5
5	21	3
6	7	7
7	7	3
8	3	1
9	2	1
10	1	2
11	4	..
12	2	..
13	2	1
14	3	1
15	3	..
16	..	1
17	..	1
21	..	1
22	2	..
23	1	..
30	..	1
Average number of days between divisions	4.34	6.12

It is thus evident that a change in environmental conditions alters the division rate in Amoeba, but does not alter the rhythmic periodicity of high and low rates. This agrees with what WOODRUFF (1917) found as to the effects of environmental changes on the periodicity of endomixis in Paramecium. Whether some process similar to endomixis occurs periodically in *Amoeba proteus* remains to be determined; further study is needed both of the rhythms themselves, and of possible accompanying cytological changes.

THE ORIGIN AND THE METHODS OF REPRODUCTION OF POLYNUCLEATE FORMS OF *Amoeba proteus*

It has generally been accepted that the polynucleate condition of an amoeboid cell is produced by the fusion of two or more mononuclear cells. STOLC (1906) observed the fusion of two sister mononucleate individuals

TABLE 6

Variation in the division rates between the individuals in two lines of the same race of Amoeba proteus when kept under the same environmental conditions. Two sister lines were kept under three different sets of culture conditions.

NUMBER OF DAYS BETWEEN DIVISIONS	NUMBER OF INDIVIDUALS THAT DIVIDED, WHEN THE CULTURE FLUID WAS RENEWED EVERY					
	Three days, in lines		Five days, in lines		Ten days, in lines	
	C_9	C_{9S}	D_{19}	D_{19S}	E_{19}	E_{19S}
1	61	66	26	27	27	9
2	58	67	46	39	28	27
3	26	48	17	27	16	11
4	19	19	8	18	6	13
5	7	27	5	18	5	9
6	12	21	5	10	4	7
7	11	11	2	6	2	4
8	5	13	2	8	3	4
9	1	7	..	1	1	5
10	7	3	2	2	1	1
11	5	6	1	3	..	..
12	1	2	4	3	1	1
13	3	2	1	1	1	2
14	1	2	1	2	..	..
15	3	4	..	1	1	1
16	2	..	..	1	1	..
17	3	..	..	..	1	1
18	2	1	..	..	..	..
19	..	2	1	1	1	..
20	..	..	..	1	2	..
22	..	..	..	1	..	..
24	..	..	..	..	1	..
25	..	..	1	..	..	..
29	1	..	..	..	..	..
30	..	..	1	..	..	..
32	..	..	1	..	..	..
39	..	..	..	..	1	..
Average interval between divisions	4.42	4.12	4.01	4.40	4.42	4.42

of *Amoeba proteus*. LAMBERT (1912) has shown that giant polynucleate cells *in vitro* are produced by the fusion of several wandering cells. Re-

cently, however, LEWIS and WEBSTER (1921), working with tissue cultures, while uncertain as to how the polynucleate cells arise, found evidence for the amitotic division of the nucleus without a division of the cytoplasm, but could find no evidence for a fusion of cells. HEGNER (1920) explains nuclear doubling in *Arcella dentata* by a failure of one nucleus and half of the cytoplasm to separate into the new shell which is formed when the parent nucleus divides. STOLC (1906) had previously observed that by an alteration in the culture medium, the binucleate form of *Amoeba proteus* could develop directly from the mononucleate condition. This occurred when the mononucleate Amoeba was placed in a culture fluid containing very little food, or an over-abundance of food; when the mononucleate Amoeba was kept for a long period in the same culture fluid; and twice in a line that was pedigreed for more than 20 months. From these observations, he concluded that the binucleate form arises from the mononucleate, by a division of the nucleus without a simultaneous division of the cytoplasm; but he did not determine how this took place.

Occurrence of polynucleate Amoebae

During the past year, forms with two, three and four nuclei have appeared in each of 18 isolation pedigree lines of *Amoeba proteus*. The frequency of their appearance varies considerably. It does not depend upon the quantity of available food material, for they may arise in cultures that have an abundance or a lack of food. The length of time that the mononucleate Amoeba is kept in a given quantity of culture fluid seems to affect the number of polynucleate forms that may appear. They rarely appear when the mononucleate organism is transferred from day to day into a fresh culture medium. However, if the transfers are made at intervals of two, three, five or ten days, the tendency for the Amoebae to become polynucleate is very strong. It was found that a constant supply of these large Amoebae could be obtained by the following procedure. An Amoeba is placed in a cavity of a hollow-ground slide which contains 0.3 cc of a dilute hay-infusion Chilomonas culture. The slide is placed in a moisture chamber. The small amount of liquid that evaporates is replaced periodically by the addition of fresh culture fluid. Under such conditions the Amoeba divides at irregular intervals, producing in a short time a large population. About thirty days after the start of the experiment, polynucleate Amoebae begin to appear. Careful examinations of the culture failed to reveal any signs of fusion of one individual with another. At about the period of the appearance of the polynucleate

forms, the total number of Amoebae in the cavity remains constant for a while, and then begins to decrease. The conditions existing in the cavity are therefore unfavorable for the multiplication of the organisms. Such unfavorable conditions may be brought about by the accumulation of the excretion products of the Amoebae and the other organisms that have populated the culture, by a concentration of the organic substances which are left behind when the slight but continuous evaporation of water occurs, or, by a combination of these changes.

Origin of the polynucleate Amoebae

Since the polynucleate Amoebae did not arise in these cultures by a fusion of cells, their origin was probably due to an irregularity in the division process of the mononucleate forms. SCHULZE (1875) has shown that an Amoeba reproduces by a simple division, the two portions moving apart until the very thin strand of protoplasm, which bridges the gap between them, finally breaks. The mononucleate forms of *Amoeba proteus* divide in the same manner, but the division of the nucleus, so clearly seen in SCHULZE'S *Amoeba polypodia*, could not be discerned in *Amoeba proteus*. In order to make intelligible the experiments later described, an account is given of the external features of fission.

The process of division may be considered as taking place in two stages:

1. *Resting stage.* The Amoeba is a spherical mass of protoplasm with short blunt pseudopods projecting from its surface in all directions (figure 3, a to c). There is a very slow movement of the protoplasm, but the Amoeba does not change its shape or position. The nucleus can not be seen in the living specimen. Whole mounts of the Amoebae in this resting stage, prepared by fixation in Yocum's fluid and stained with iron haematoxylin indicate that the nucleus has become completely disorganized. Spherules of chromatin material are scattered throughout the cytoplasm. I have not determined whether the absence of the nuclear membrane is due to the destructive action of the fixing agent, or whether it is characteristic of the method of nuclear division.

2. *Cytoplasmic division.* The Amoeba begins to elongate by a slow streaming of the protoplasm, which flows first in one direction, then in the opposite direction. As the Amoeba elongates, the central portion becomes narrower than the parts on either side of it (figure 3, d to h). Pseudopods radiate from each of the dividing parts. They are projected for a short distance only. Some of these are withdrawn and larger ones move out. This process of pushing out a pseudopod and then withdrawing it, continues throughout the division of the cytoplasm. The movement is not

limited to the pseudopods. It is seen in the constricted central portion which becomes a narrow strand of protoplasm. For several minutes there is an interchange of protoplasmic materials between the two dividing parts through this connecting strand. At first there is a slow movement of granules from A to B, (figure 3, d to h), which stops, and then the streaming proceeds from B to A. This alternating movement continues as

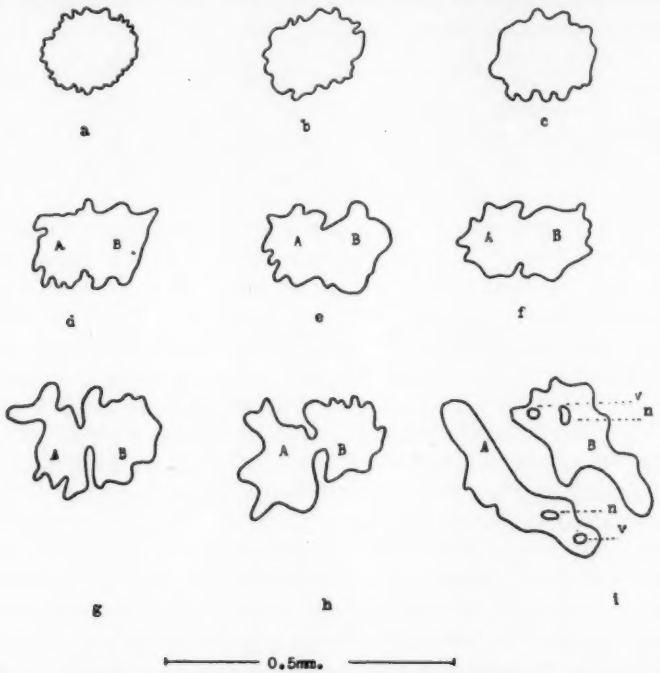

FIGURE 3.—Camera lucida sketches of successive stages in the division of a mononucleate individual of *Amoeba proteus*. a, b, c, stages in the quiescent period, during which nuclear division occurs; a, at 10:30 a.m.; b, 10:35 a.m.; c, 10:45 a.m.; d to i, stages in the division of the cytoplasm, with the separating parts marked A and B; n, nucleus; v, vacuole; d, at 10:47 a.m.; e, 10:50 a.m.; f, 10:53 a.m.; g, 10:56 a.m.; h, 11:00 a.m.; i, 11:15 a. m.

the parts move away from each other, and ceases when the strand between them comes to be too narrow to permit the passage of even the smallest granules. The connection, now consisting almost entirely of ectoplasm, breaks, and two mononucleate individuals are the result (figure 3, i).

The process of cytoplasmic division is of long enough duration (15 to 30 minutes) to permit of experimental study. Can the process in any way be

altered so as to prevent the cytoplasm from dividing? It was found that the alternating streaming of the protoplasm, which is so characteristic of the division process, could be modified by local application of certain chemicals and even by a mechanical stimulus applied to several of the short pseudopods. The pseudopods invariably moved away from the stimulus, and by the continued application of it, the movement of the protoplasm was made to proceed more in one direction (away from the source of stimulation) than in the other. It was possible to stop the alternation in the streaming of the protoplasm during the cytoplasmic division and to force the streaming to proceed in one direction only by the following method: A small drop (diameter 1 mm) of culture fluid is placed on a glass slide; a dividing Amoeba is transferred with a fine capillary pipette to the outer edge of this drop so that only a portion of the added fluid merges with the drop. The organism is so oriented that one of the dividing parts, B (figure 3, d), fits snugly in the space enclosed by the minute quantity of liquid that was transferred with the Amoeba. The other portion, A, projects into the larger drop. The movement of the protoplasm alternates through the connecting strand for a few minutes. When a pseudopod of the part B reaches the edge of the drop, it is quickly withdrawn. As the drop evaporates, its edge approaches B, and prevents it from projecting any more pseudopods. The only part of B that is not affected by the evaporating drop is the portion attached to the connecting strand. Further evaporation of the drop that surrounds B forces its protoplasm to flow with increased rapidity into A. This streaming of one part into the other continues as long as the movement of B in any other direction is prevented. Eventually all of the part B flows into A, and the resultant Amoeba does not attempt to divide again. An organism is produced which is identical in both morphological and physiological characteristics with the binucleate Amoebae found in the isolation cultures, and in the material collected from ponds. The trinucleate and the tetranucleate Amoebae can be produced by the same method.

Since the nucleus divides while the Amoeba is at rest, (figure 3, a to c) the polynucleate form is produced by a failure of the cytoplasm to divide with it. The process of cytoplasmic division begins, but is prevented from going to completion by a mechanical or a chemical condition existing in the culture medium. Such conditions are more frequent in old concentrated cultures than in freshly prepared, dilute ones.

Methods of reproduction of polynucleate Amoebae

The question as to the permanence of these nuclear modifications leads to a study of the methods of reproduction of the polynucleate forms.

STOLC (1905) made an extensive investigation of this problem. In an experimental study on 62 binucleate Amoebae, 5 trinucleate Amoebae, and 1 tetranucleate Amoeba, he found that the methods of division of protoplasm in the polynucleate condition could be classified as follows:

1. Protoplasmic division without a simultaneous division of the nuclear material.
2. Protoplasmic division with a simultaneous division of all the nuclei.
3. Protoplasmic division with a simultaneous division of only part of the total nuclei.

Furthermore, he described, but did not classify, a case in which a binucleate Amoeba became tetranucleate, apparently by a division of the nuclei without a division of the cytoplasm.

Division of binucleate Amoebae

Of the 248 binucleate Amoebae isolated for the present investigation, 11.3 percent, after living in the culture medium for an average period of 10 days, died. This mortality among the binucleate forms is significant when compared with that of only 5 percent among the several hundred mononucleate Amoebae that were isolated. The method of division of the nuclei and of the cytoplasm, and the distribution of the nuclei to the cytoplasm occur in a number of different ways:

1. *Division of the cytoplasm without a division of the nuclei.* In 13 cases out of the total 248, or in 5.2 percent, the Amoeba existed in the binucleate condition for an average period of 8.2 days, and then divided into two large Amoebae, each of which was mononucleate. These continued to reproduce by an ordinary division into two parts, the products being always mononucleate.

2. *Division of the cytoplasm with an incomplete amitotic division of the nucleus.* In 3.2 percent of the cases, the Amoeba, after existing in the binucleate condition for an average period of 5.5 days, divided into two parts, each of the products being mononucleate. These nuclei, however, instead of being smooth discoids, as were those of the parent, were bent or folded. The amount of the abnormality in the shape of the nucleus varied from a small protuberance at one end to a complete folding of the nucleus on itself. In the latter condition, two nuclei seemed to be joined together, or conjugating. But that this is not the case is shown at the next division, the products of which continued to be two Amoebae, each with a bent nucleus. One line was pedigreed for 35 generations, and a race of Amoebae with a tendency to possess bent nuclei was produced. Some of the members of this line had normal nuclei, but the majority possessed bent nuclei.

These Amoebae are large,—about 1.5 times the size of an Amoeba with a smooth nucleus. They are less viable and have a slower rate of division than the forms with normal nuclei. It is assumed that this condition is brought about by an incomplete amitotic division of the nucleus. In a recent publication, TAYLOR (1923) finds cytological evidence which indicates that an incomplete amitotic division of the nucleus occurs in *Amoeba proteus*.

3. *Division of both nuclei without a division of the cytoplasm.* In 4 cases, or 1.71 percent of the total number of binucleate Amoebae that were isolated, examination after about five days showed that the organism contained four small nuclei instead of the two large ones that were present upon the previous examination. There had evidently taken place in the interim, a division of the nuclei without a division of the cytoplasm.

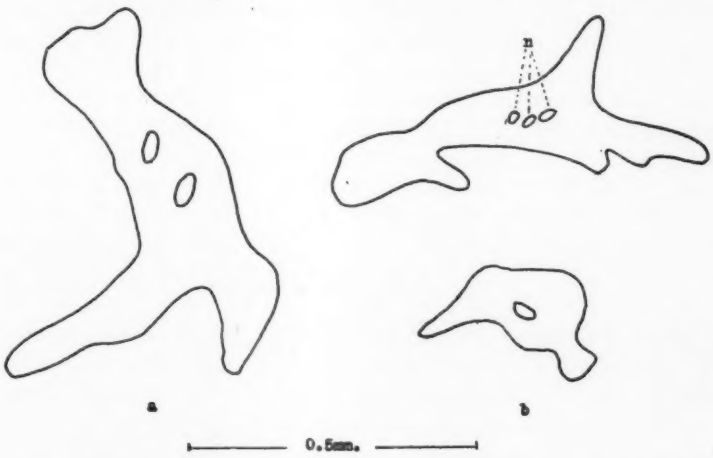

FIGURE 4.—Camera lucida sketches of the division of a binucleate Amoeba into a larger and smaller portion, the former with 3 nuclei (n), the latter with but 1. a, parent, about an hour before division; b, progeny, about an hour after division.

4. *Division of the cytoplasm with a division of both nuclei.* (a) In 5.7 percent of the cases, the Amoeba, after existing in the binucleate condition for an average period of 8.4 days, divided into 2 binucleate Amoebae.

(b) In 4.5 percent of the cases, the Amoeba, after existing in the binucleate condition for an average period of 8.7 days, divided into two unequal parts; the smaller one being mononucleate, the larger portion trinucleate (figure 4).

(c) In 138 cases, or 55.6 percent the Amoeba, after existing in the binucleate condition for an average period of 6.3 days, divided into three Amoebae, the largest one being binucleate, the two smaller ones mononucleate (figure 5). This, as the figure shows, occurred by a direct division

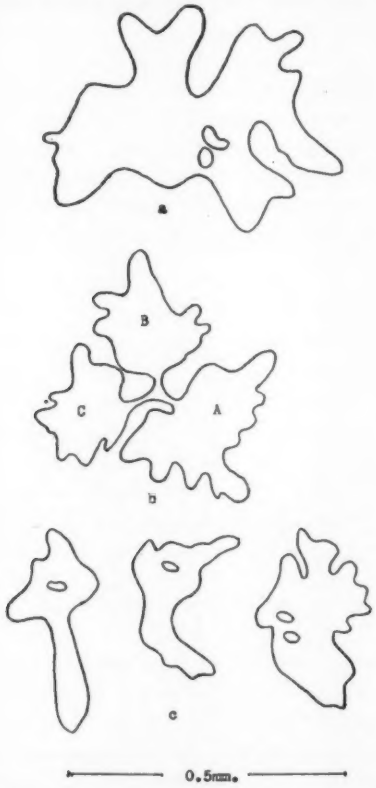

FIGURE 5.—Camera lucida sketches of the division of a binucleate Amoeba into three,—two each with one nucleus, the third with two nuclei. a, one day before division. b, during division, with separating parts marked A, B, C; c, progeny, 25 minutes after division.

into three parts, confirming the earlier observations of STOLC (1905). One of the mononucleate parts was the first to break away from the connecting strand; the remaining two parts separated within the next minute.

(d) In 32 cases, or 12.9 percent of the total 248, the Amoeba after existing in the binucleate condition for an average period of 7.3 days,

divided into four mononucleate Amoebae. Although a simultaneous division into four parts was not observed, it is probable that this is what actually occurred.

Resumé

The most common method of reproduction of the binucleate Amoebae is that in which a division of the cytoplasm and both of the nuclei occurs. 78.6 percent of the 248 binucleate forms divided in this manner. Two general classes of divisions can be distinguished in this group, depending upon the rates of division of the two nuclei. The nuclei may divide simultaneously, or the division of one may lag behind the division of the other. The production of four mononucleate Amoebae, by a direct division, which occurred in but 16 percent of the 195 cases in this category, is probably the result of a simultaneous division of the nuclei. More than 70 percent of the remainder produced, by a direct division into three parts, one binucleate and two mononucleate Amoebae. This method of division is probably brought about by a slight difference in the rate of division of the two nuclei. One of the nuclei divides, producing a trinucleate Amoeba, which immediately begins to separate into three unequal mononuclear parts. During the triple division of the cytoplasm, the other nucleus divides, but a further division of the cytoplasm is prevented by the one already in progress. The products of such a division, if permitted to go to completion, are one large binucleate form, and two smaller mononucleate forms.

Experimental modification

This process, however, can be modified by the use of the evaporating-drop method (page 141). While the triple cytoplasmic division is in progress (figure 5, b) the parts which will eventually form the binucleate portion, A, and the mononucleate portions, B and C, can be identified by their differences in size. If the dividing Amoeba is so oriented in a minute drop that the mononucleate part, B, can not send out its pseudopods, the streaming of its protoplasm can proceed in only one direction, through the connecting strand into either A or C. If it flows into the mononucleate part, C, division into two binucleate Amoebae results. If, on the other hand, the protoplasm of B flows into the binucleate part, A, again a division into two Amoebae occurs, one of which is mononucleate, the other trinucleate (figure 4). The frequency with which these two methods of division occur under natural conditions is approximately the same. Finally, the protoplasm of both the mononucleate portions, B and C, can be made to flow into the binucleate part, A, causing the production

of a tetranucleate Amoeba. This occurs in very few cases. Although these experiments were modified in various ways, no other methods of division than those already described could be obtained. It seems, therefore, that the trinucleate and the tetranucleate Amoebae originate, like the binucleate, by conditions that prevent the process of division of the cytoplasm from going to completion.

Division of the trinucleate Amoebae

Only 22 trinucleate Amoebae were available for the study of the methods of reproduction of these forms. Five of these Amoebae did not divide, but disintegrated, having lived in the culture medium for an average period of 29.4 days. The length of life (from the date of origin to disintegration) of a trinucleate Amoeba is therefore about three times that of a binucleate form, and nearly six times that of a mononucleate Amoeba. The mortality is also greater than that of the binucleate and mononucleate forms. The methods of division of the trinucleate Amoebae are even more variable than are those of the binucleate Amoebae:

1. *Division of the cytoplasm with the division of only one of the nuclei.* In one case, the Amoeba existed in the trinucleate condition for 8 days. It then divided into two binucleate Amoebae. Each of the parts contained one large undivided nucleus and one of the products of the divided nucleus.

2. *Division of the cytoplasm with the partial division of only one of the nuclei.* In another case, the Amoeba existed in the trinucleate condition for 8 days. It then divided into two Amoebae. One of the parts was mononuclear, the other contained one smooth and one bent or folded nucleus.

3. *Division of the cytoplasm with a division of all the nuclei.* (a) In one case, the Amoeba existed in the trinucleate condition for 4 days. It then divided into three unequal binucleate Amoebae.

(b) In three cases, the Amoeba existed in the trinucleate condition for an average period of 7 days; it then divided into three Amoebae, one trinucleate, one binucleate and one mononucleate (figure 6).

(c) In two cases, the Amoeba existed in the trinucleate condition for an average period of 15 days; it then divided into three parts, two of which were mononucleate, the third was a large tetranucleate Amoeba.

(d) In six cases, the Amoeba existed in the trinucleate condition for an average period of 7.3 days; it then divided into four parts. The largest portion was trinucleate, the three smaller Amoebae were mononucleate.

(e) In another case the Amoeba existed in the trinucleate condition for 5 days; it then divided into four parts. The larger two were binucleate, the other two were each mononucleate.

(f) In two cases the Amoeba existed in the trinucleate condition for an average period of 7 days; it then divided into five Amoebae. Four of these were mononucleate; the other was binucleate.

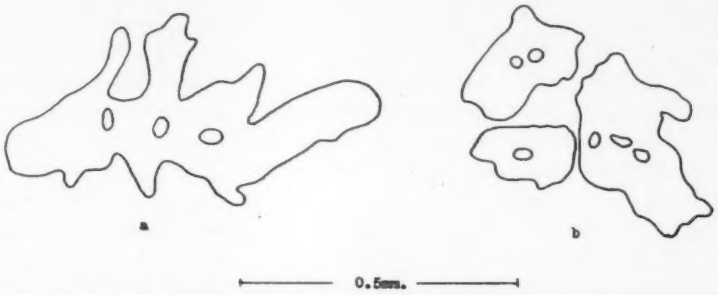

FIGURE 6.—Camera lucida sketches of the division of a trinucleate Amoeba into three progeny, containing respectively 1, 2 and 3 nuclei. a, parent 3 days before division; b, progeny about an hour after division.

Resumé

Thus the most common method of reproduction in the trinucleate Amoebae is again a division of the cytoplasm accompanied by a division of all the nuclei. 68.2 percent of the 22 trinucleate Amoebae that were isolated divided in this manner. The great variation in the number of cytoplasmic products, and the distribution of the daughter nuclei to them is probably determined by the differences in the rates of division of the three parent nuclei. The three nuclei may divide simultaneously; two of them may divide together; or, all three may divide at different times. The tendency seems to be for two of the nuclei to divide simultaneously. A pentanucleate Amoeba would thus be produced and it will immediately begin to divide into five parts. While the cytoplasmic division is in progress, the third of the parent nuclei divides, but a further division of the cytoplasm does not occur. The products of such a division would be four mononucleate and one binucleate Amoeba. This has actually occurred in only two of the cases. In the majority of the cases (80 percent of the 15 divisions described in group 3) the cytoplasm divides into only three or four parts, and generally at least one of these portions is either trinucleate or tetranucleate. There are, therefore, indications that processes similar to those going on during an evaporating-drop experiment, modify the

method of division of the trinucleate Amoebae. The observations, which were limited to the divisions of but 17 organisms, represent but a small proportion of the total possible methods of reproduction of the trinucleate Amoebae. There are many more, depending upon the nature of the nuclear division, the number of nuclei that divide, and their relative rates of division, the number of cytoplasmic products, and the distribution of the nuclei to these parts.

Division of the tetranucleate Amoebae

Five out of the eight tetranucleate Amoebae that were isolated died, having lived in the culture medium for an average period of 19.8 days. The remainder divided in an irregular manner:

(1) *Division of the cytoplasm without a division of any of the nuclei.* In one case, the Amoeba existed in the tetranucleate condition for 1 day. It then divided into one large mononucleate Amoeba and a still larger trinucleate form.

(2) *Division of the cytoplasm with a division of only one of the nuclei.* In another case, the Amoeba existed in the tetranucleate condition for 14 days. It then divided into one trinucleate and two mononucleate Amoebae.

(3) *Division of part of the nuclei without a division of the cytoplasm.* Lastly, an Amoeba existed in the tetranucleate condition for 8 days. It was now found to contain 6 nuclei. Two of the four nuclei of the parent had divided but the cytoplasmic division had been prevented, resulting in the production of a hexanucleate Amoeba. The latter organism disintegrated after a length of life of 22 days.

The rarity of appearance of the tetranucleate Amoeba, and the high mortality (62.5 percent amongst those isolated), make an adequate study of the method of reproduction in these forms extremely difficult.

SUMMARY

1. The granular Amoebae (*Amoeba proteus* according to SCHAEFFER) and the raptorial Amoebae (*A. dubia* SCHAEFFER), pedigreed for 40 generations, were found to retain, throughout, their differential characteristics (confirmation of SCHAEFFER and others).

2. In the raptorial Amoebae (626 cases) the average period between fissions was 1.6 days. In the granular Amoebae (846 cases) it was 2.5 days.

3. In all the lines studied, the reproduction was rhythmic, periods of high reproductive rate alternating with periods of low reproductive rate,

as in infusoria. This seems to indicate that some process akin to endomixis occurs periodically in Amoeba.

4. The rates of division of sister lines of the same race of *Amoeba proteus* when kept under the same environmental conditions vary; but these variations are less than those between different races kept under the same conditions, and less than those between lines of the same race kept under different conditions.

5. An abnormal (bent) condition of the nucleus has a considerable lowering effect on the division rate, which persists even after the normal condition of the nucleus has returned.

6. The division rate and the vitality of the organism are lowered, when the Amoebae are kept for long periods in the same culture fluid. The optimum conditions for reproductive activity were obtained by daily transfers.

7. Four sets of long-continued experiments show that the rhythmic character of the divisions in *Amoeba proteus* is similar for all the races that were studied, both under the same and under different environmental conditions.

8. A change in the environmental condition, therefore, affects the division rate, but not the periods of high and low reproductive activity. This is similar to the condition found in infusoria.

9. Polynucleate Amoebae appeared in isolation pedigree cultures of *Amoeba proteus*. They also appeared in mass cultures upon certain modifications of the culture medium.

10. There is no evidence to show that the polynucleate forms arise by a fusion of mononucleate cells. The evidence indicates that there has been a division of the nucleus without a division of the cytoplasm.

11. An experimental method is presented by which it is possible to prevent or modify the division of the cytoplasm, and thus to cause the production of polynucleate Amoebae. It is suggested that something similar to this is the method of origin of these forms in nature and in laboratory cultures.

12. An extensive study was made of the methods of division in polynucleate Amoebae, based upon observation of 248 binucleate, 22 trinucleate and 8 tetranucleate individuals. In general this confirmed the earlier observations of STOLC (1905), but yielded certain methods not observed by STOLC. These very numerous ways of dividing are all modifications of the following:

(a) Division of the cytoplasm with incomplete division of all the nuclei.

(b) Division of the cytoplasm with incomplete division of only part of the nuclei.

(c) Division of all the nuclei without a division of the cytoplasm.

(d) Division of part of the nuclei without a division of the cytoplasm.

13. An increase in the number of nuclei is accompanied by a decrease in the division rate. An increase in the number of nuclei is accompanied also by an increase in the mortality rate.

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INTERRELATIONS OF GENETIC FACTORS IN BARLEY

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INTRODUCTION

The assignment of genetic factors to definite loci in the hereditary mechanism has been a great step in the progress of biology. While this result has been most brilliantly and extensively achieved in Drosophila, confirmation has been found in many other animals and in plants. Genetical investigations in maize, for instance, have resulted in the identification of over ninety genes and the arrangement of twenty-five of these into six linkage groups (HUTCHISON 1922).

Barley is about as favorable as maize for such studies, owing to the existence of a large number of heritable variations; and it has an advantage in that for purposes of experimental breeding it can be raised in the field twice as fast as maize. The smaller number of chromosomes in barley ($n=7$) as compared with that in maize ($n=10$) also gives barley an advantage, as the number of chromosomes corresponds with the number of linkage groups, according to the chromosome theory of heredity, and the establishment of linkage groups is relatively easy in species with a small number of such groups. The only serious drawback in genetic work with

barley lies in the excessive amount of labor required in artificial pollination which renders back-crossing impracticable and thus necessitates the study of a large number of individuals to attain a fair degree of accuracy, especially in cases of strong repulsion (HALDANE 1919).

Studies on the inheritance of barley undertaken by previous investigators have elucidated the behavior of numerous simple Mendelian characters, and some linkage relations have been reported by UBISCH (1919), MIYAKE and IMAI (1922) and NILSSON-EHLE (1922). It is the purpose of this study to reveal more interrelations between the genetic factors of barley and prepare the way for further work of the same nature.

MATERIAL AND METHODS

The strains of barley used as parents in the crosses have come from different sources, and nearly all of them were transferred to the writer by Professor R. E. CLAUSEN. Their homozygosity with respect to the characters under investigation is known, as all of them have been grown and studied at the Agricultural Experiment Station at Berkeley for two or more seasons.

The hybrid progenies as well as the parental strains were grown in the field. The work in artificial pollination was carried out in the usual manner, which need not be described here. In nearly all cases the spikes from each individual F_1 and F_2 plant were harvested and stored in an envelope bearing the pedigree number, for laboratory study, while the examination of all the F_3 plants was made in the field.

The first crosses, from which most of the data herein presented were derived, were made in the fall of 1920, and most of these were carried to the F_3 generation. The crosses made in the spring of 1922 have been carried only to the F_2 generation. On account of the limited time several characters had to be dropped out of consideration during the course of the investigation.

In testing the goodness of fit PEARSON'S χ^2 method (PEARSON 1900) is used, and the value of probability, P, is found from ELDERTON'S table. While this mathematical test is undoubtedly a great help in making decisions, weak linkage relations cannot be detected with certainty with a relatively small F_2 population. For instance, with an F_2 population of about a hundred individuals the existence of linkage with a crossover value of forty percent or more will not *per se* make the size of χ^2 significantly too great for the typical Mendelian dihybrid. In such a case the linkage value will be calculated and the size of χ^2 for the assumed linkage relation noted. The probability of linkage is increased if χ^2 for the assumed linkage relation is very small. But too much reliance has not been placed on this

procedure; for it is realized that the data of many of the actual cases of independent assortment can be calculated to show a low linkage intensity with χ^2 smaller than that for independent assortment.

While the data from a single F_2 population may not be conclusive as to linkage relations, further evidence may be secured from other crosses or from F_3 tests. On account of limited facilities it has not been practicable to grow a large number of plants for each F_3 population in these experiments; and the one outstanding difficulty encountered in working with small F_3 populations and weak linkage has been the uncertainty in classifying the populations. Among the progenies of F_2 individuals heterozygous for both factors in the same linkage group, the proportion of populations with the reversed linkage relation to those with the original linkage relation is $c^2 : (1-c)^2$, where c is the amount of crossing over expressed as a decimal fraction. When c is large, populations with the reversed linkage relation are expected to occur frequently. At the same time, it is often difficult to be sure whether a given population represents the original or the reversed linkage relation, for the fluctuations in a small population often make linkage resemble independent assortment and may even make an actual case of linkage in one direction resemble linkage in the opposite direction.

In case the majority of the populations under consideration can be classified with a fair degree of safety, the few doubtful populations are classified in these studies according to whatever slight amount of coupling or repulsion they display. In doing this the writer is fully aware of the possibility of mistakes; but this is the only way of analyzing the F_3 results and comparing them with the assumed relations worked out from the F_2 data, as the F_3 populations taken individually are too small and therefore unreliable for such comparison. The occasional misplacement of one or two populations is not a serious matter when one is aware of such a possibility, as it simply increases the apparent amount of crossing over displayed by the class into which it has been misplaced, and the cross over value displayed by the other class is correspondingly reduced. Such mistakes may be detected from the inequality of crossover values calculated for the class showing original linkage and for that showing the reversed linkage, though there is no definite way of correcting them.

Besides using YULE'S coefficient of association with a table of calculated corresponding linkage values as suggested by COLLINS (1912), formulae for the calculation of linkage values directly from the F_2 data have been worked out by EMERSON (1916) and HALDANE (1919). Professor CLAUSEN has suggested a method (unpublished) for the calculation

of linkage values which is more convenient than the others in use in conjunction with the χ^2 test for the goodness of fit. The method consists essentially of obtaining a balanced linkage ratio from the given data so that the square root of the double recessive class reveals the gametic ratio. The balancing of the ratio depends on the principle that theoretically the amount by which the different zygotic classes in a linkage distribution differ from the corresponding classes in independent assortment remains constant. For, assuming the gametic series to be

$$(m+a)AB : (m-a)Ab : (m-a)aB : (m+a)ab,$$

the zygotic series is:

$$\begin{aligned} AB &= 9m^2+(2ma+a^2) \\ Ab &= 3m^2-(2ma+a^2) \\ aB &= 3m^2-(2ma+a^2) \\ ab &= m^2+(2ma+a^2) \end{aligned}$$

Assuming the gametic series to be

$$(m-a)AB : (m+a)Ab : (m+a)aB : (m-a)ab,$$

the zygotic series is:

$$\begin{aligned} AB &= 9m^2-(2ma-a^2) \\ Ab &= 3m^2+(2ma-a^2), \\ aB &= 3m^2+(2ma-a^2) \\ ab &= m^2-(2ma-a^2) \end{aligned}$$

The method suggested by Professor CLAUSEN is employed in this paper and will be indicated at the first instance of calculation of linkage value. Since the value calculated by this method does not differ from that calculated by HALDANE's method, HALDANE's formula for the probable error of linkage value is here used. The formula is

$$\pm .477\sqrt{\frac{(2+p^2)\ (1-p^2)}{(1+2p^2)\ n}}$$

where n is the total number of individuals under consideration, p is the crossover value in case of repulsion, and $1-p$ is the cross over value in case of coupling.

A consideration of the size of the probable error is a guide to the significance of the difference in linkage values and another means of ascertaining the existence of weak linkage. With respect to the latter function the use of the probable error is subject to the same limitations as encountered in the use of χ^2, as the difference between the calculated linkage value and the value for independent assortment may not exceed the probable error of the former (or the latter[1]) so much as to be significant.

[1] The probable error for independent assortment (i.e., with P equal to 0.5) and χ^2 for independent assortment are both useful in ascertaining what calculated amount of linkage intensity may be considered as significant.

THE MULTIPLE ALLELOMORPH SERIES A, a^3, a^d

The lateral floret of barley exhibits four main grades of fertility upon which HARLAN (1918) has classified the four species of barley (which are probably better ranked as sub-species of *Hordeum sativum* Jess.); namely,

(1) *Hordeum vulgare* — lateral floret fully fertile;
(2) *Hordeum intermedium*[2] — lateral floret partially fertile;
(3) *Hordeum distichon* — lateral floret staminate;
(4) *Hordeum deficiens* — lateral floret rudimentary.

That 1, 3 and 4 form a multiple-allelomorph series was first pointed out by ENGLEDOW (1920), based on the investigations of BIFFEN (1906, 1907) and his own. Data obtained from the present investigation confirming this case of multiple allelomorphism are assembled in tables 1, 2 and 3.

TABLE 1

Hordeum vulgare × *H. distichon or reciprocal.*

NUMBER OF F_2 CULTURE	NON-*vulgare*	*Vulgare*
22215	15	10
23215	194	56
22216	41	6
22219	28	13
23219	154	47
22221	10	4
23221	63	21
22224	22	4
23224	87	25
22228	135	53
23228	73	26
22235	66	19
23235	107	27
*21201	34	11
*21202	38	16
*21208	27	13
Total	1094	351
Calculated	*1083.75*	*361.25*

Deviation, 10.25 ± 11.10

The prefixes 21, 22, 23 indicate the years 1921, 1922 and 1923, respectively, when the plants were grown.

224 and 219 are from reciprocal crosses of the same parents.

*Cross made by Professor R. E. CLAUSEN.

[2] HARLAN and HAYES (1920) have shown that *H. intermedium* may be secured as a recombination product by crossing *H. distichon* with a certain type of *H. vulgare*.

TABLE 2

Hordeum vulgare × *H. deficiens or reciprocal.*

NUMBER OF F_2 CULTURE	NON-*vulgare*	*Vulgare*
22226	39	12
23226	54	22
23236	172	83
156×205	162	58
Total	427	175
Calculated	*451.5*	*150.5*

Deviation 24.5 ± 7.17

TABLE 3

H. distichon × *H. deficiens.*

NUMBER OF F_2 CULTURE	NON-*distichon*	*Distichon*
22217	71	33
23217	234	69
Total	30	102
Calculated	*305.25*	*101.75*

Deviation, 0.25 ± 5.89

If the assumption of multiple allelomorphism is correct, crossing any two of these forms should not give rise to the third form. The fulfillment of this requirement is at once clearly seen in the *vulgare*×*distichon* and *distichon*×*deficiens* crosses. In the *vulgare*×*deficiens* crosses the lateral floret of the F_1 is similar to that of *H. distichon*. But all such forms in F_2 were found to be heterozygous, and hence are genetically distinct from the true-breeding *H. distichon*. Data supporting this statement are recorded by BIFFEN (1907) and are furnished by this study. Thirty-six F_2 plants of culture 22226 were carried to the F_3 generation. Of these the seven *vulgare* plants bred true; so did all the fourteen plants of the *deficiens* type. The fifteen intermediate plants similar to *H. distichon* all bred like the F_1.

The symbols A, a^s, and a^j are here used to indicate the factors for *deficiens*, *distichon* and *vulgare*, respectively.

CROSSES INVOLVING A a^s a^j AND L l

The rachilla of the barley spike is covered either with long and unbranched hairs or with short branched hairs. This difference in the type

of hair on the rachilla has long been made use of in distinguishing otherwise closely similar strains of barley, and the mode of inheritance resulting from crossing the two types has been reported by UBISCH (1916) and ENGLEDOW (1920) as a case of monohybrid with the long-hair character dominant.

Letting *L l* denote the pair of factors concerned in this case, table 4 indicates independent assortment between the *L l* pair and the *A* a^s a^f series. The populations in which the two dominants have entered from

TABLE 4

NUMBER OF CULTURE	PARENTAL COMBINATIONS	F_2 CLASSES			
		La^s	*La*^{f}	*la*^{s}	*la*^{f}
22216	*la*^{f} and *La*^{s}	28	6	13	0
22219	*la*^{f} and *La*^{s}	21	9	7	4
23219	*la*^{f} and *La*^{s}	117	37	37	10
22224	*La*^{s} and *la*^{f}	16	4	6	0
23224	*La*^{s} and *la*^{f}	52	12	13	9
22228	*La*^{s} and *la*^{f}	94	40	41	13
23228	*La*^{s} and *la*^{f}	54	19	19	7
22221	*La*^{f} and *la*^{s}	9	3	1	1
23221	*La*^{f} and *la*^{s}	49	15	14	6
140×136	*La*^{s} and *la*^{f}	68	16	19	9
23236		*LA*	*La*^{f}	*lA*	*la*^{f}
23236	*La*f and *lA*	128	62	38	19
		LA	*La*^{s}	*lA*	*la*^{s}
22217	*La*^{s} and *lA*	34	25	17	8
23217	*La*^{s} and *lA*	183	52	51	17
22218	*lA* and *La*^{s}	7	5	2	1
Total.....		860	305	278	104

For independent assortment, $\chi^2 = 1.94$; P = 0.586

one side and the two recessives from the other, give totals of 450 : 143 : 155 : 52, while the populations in which each side has contributed one dominant and one recessive give totals of 410 : 162 : 123 : 52. Thus there can be no objection that the summation of the two groups of populations has obscured a linkage relation.

CROSSES INVOLVING *L l*, *A* a^s a^f AND *N n*

In most cultivated varieties of barley the caryopsis is adherent to the lemma and palea at maturity, but many races exist in which the caryopsis

remains free. The distinction is due to a single factor difference with the adherent (hulled) condition completely or partially dominant over the free (naked) condition (BIFFEN 1907, etc.). Using *N n* to denote the pair of factors concerned, table 5 indicates independent assortment between *L l*, *A* a^s a^f and *N n*.

TABLE 5

CULTURE	PARENTAL COMBINATIONS	F_2 CLASSES							
		La^s*N*	*La*^{s}*n*	*La*^{f}*N*	*La*^{f}*n*	*la*^{s}*N*	*la*^{s}*n*	*la*^{f}*N*	*la*^{f}*n*
22221	*La*^{f}*n* and *la*^{s}*N*	3	6	1	2	0	1	1	0
23221	*La*^{f}*n* and *la*^{s}*N*	38	11	12	3	10	4	3	3
		LAN	*LAn*	*La*^{f}*N*	*La*^{f}*n*	*lAN*	*lAn*	*la*^{f}*N*	*la*^{f}*n*
23236	*La*^{f}*n* and *lAN*	98	26	45	11	27	10	13	8
Total		139	43	58	16	37	15	17	11

For independent assortment, $\chi^2 = 11.54$; $P = 0.118$

CROSS INVOLVING *L l*, *S s*, *N n* AND *A* a^s

The *L l* pair, besides having effect on the rachilla as mentioned before, also determines the nature of pubescence of the outer glumes in a similar way. But the extent of the pubescence of the outer glumes is determined by another factor pair identified from the following cross, which also yields results concerning its relations with *A* a^s, *L l* and *N n*.

Number 154 has the genetic constitution[3] *L L* a^s a^s *N N*, with the pubescence of the outer glumes restricted to the central ridge; number 025 has the genetic constitution *llAANN*, with the major portion of the outer glumes covered by short hairs. On crossing No. 154 ♀ with No. 025 ♂ the F_1 plants possessed outer glumes in a heavily haired condition not found in either parent. The distribution of the F_2 plants with respect to hairiness is as follows:

237 plants,—outer glumes heavily haired and rachilla long-haired;

77 plants,—central ridge of outer glumes haired and rachilla long-haired;

[3] The lateral floret of this strain is much less well developed than that of the typical *H. distichon*. The breeding results reveal that it has the formula $a^s a^s$, the modification being due to genetic factors lying outside the locus of the triple-allelomorph series.

72 plants,—outer glumes covered with short hairs and rachilla short-haired;

21 plants,—central ridge of outer glumes haired and rachilla short-haired.

The actual distribution fits closely with the typical dihybrid ratio. Thus, if we denote the factor pair affecting the extent of pubescence on the outer glumes by *Ss*, No. 154 had the formula *LLss*, No. 025 had the formula *llSS*, and F_1 had the formula *LlSs*. *S* is only partially dominant over its allelomorph in the spreading effect, while *ss* restricts the hair to the central ridge.

The distribution with respect to all four pairs of contrasting characters is as follows:

LSNA	147	*lSNA*	44
LSNa^s	58	*lSNa*^{s}	18
LSnA	26	*lSnA*	6
LSna^s	6	*lSna*^{s}	4
LsNA	36	*lsNA*	12
LsNa^s	8	*lsNa*^{s}	2
LsnA	28	*lsnA*	6
Lsna^s	5	*lsna*^{s}	1

If we disregard the *Nn* pair for the moment, it can be seen that the remaining factors are assorted in conformity with the typical Mendelian trihybrid ratio. The trihybrid distribution is as follows:

LSA	173
LSa^s	64
LsA	64
Lsa^s	13
lSA	50
lSa^s	22
lsA	18
lsa^s	3

For independent assortment
$\chi^2 = 6.85$; $P = 0.445$

The relation between *Ss* and *Nn* is represented by the distribution 267 *SN* : 42 *Sn* : 58 *sN* : 40 *sn*, which clearly indicates linkage. The linkage value is calculated by Professor CLAUSEN'S method, in table 6. The size of χ^2 is somewhat too large. The disturbing element is due to the large deviation from the 3 *N* : 1 *n* ratio, which is 3.36 times the probable error. This is merely an extreme case that occasionally happens in random sampling without invalidating the monohybrid nature of hulled *versus* naked. This statement finds confirmation in the F_3 data.

TABLE 6

PHENOTYPE	OBSERVED FREQUENCY	CALCULATED FREQUENCY INDEPENDENT ASSORTMENT	OBSERVED MINUS CALCULATED		BALANCED LINKAGE RATIO
SN	267	228.9375	38.0625	228.9375+31.3125	=260.25
Sn	42	76.3125	−34.3125	76.3125−31.3125	= 45.00
sN	58	76.3125	−38.3125	76.3125−31.3125	= 45.00
sn	40	25.4375	14.5625	25.4375+31.3125	= 56.75
Total	407		125.2500 ÷ 4 = 31.3125		

Percent of crossing over $= 100\left(1-2\sqrt{\frac{56.75}{407}}\right) = 25.32 \pm 1.73$

For the calculated linkage relation, $\chi^2 = 9.05$; $P = 0.029$

Table 7 gives the distribution of characters in the fifty F_3 populations of this cross. When all the F_3 populations in which there is segregation with respect to the adherence of the caryopsis are summarized, the distribution is 635 hulled : 228 naked, which is a fairly close agreement with theoretical expectation.

Table 8 gives the distribution in the eleven F_3 populations grown from the F_2 plants heterozygous for both *S* and *N*. It is probable in this case that cultures 23295, 23313, and 23289 are cases of reversed linkage, while in the remaining eight cultures the original linkage relation is exhibited. The eight cultures together give a distribution of 171 *SN* : 36 *Sn* : 30 *sN* : 41 *sn*. The crossover value calculated for this distribution is 27.54 ± 2.05 percent. The three cases of reversed linkage give a total distribution of 54 *SN* : 31 *Sn* : 20 *sN* : 3 *sn*. The crossover value calculated from this distribution is 23.58 ± 5.68 percent. Both of these crossover values do not differ significantly from the value determined from the F_2 data, which is 25.32 percent.

DENSITY OF THE SPIKE IN RELATION TO *L l*, *S s*, *N n*, *A a*^{1}

Linkage between the factor concerned in the adherence of the caryopsis and a factor for density with a gametic ratio of "5 : 1" has been reported by UBISCH (1921). MIYAKE and IMAI (1922) report the linkage of *Nn* with two factors for density with crossover values 13 percent and 23 percent, respectively. To determine the relation between density and the other characters dealt with in the cross 154×025, measurements have been taken on the ten internodes above the first node giving rise to a good central spikelet. The best spike is chosen for measurement in each plant.

TABLE 7

CULTURE NUMBER	CHARACTER OF F_2 PARENT	F_3 DISTRIBUTION															
		LSNA	*LSNa*	*LSnA*	*LSna*	*LsNA*	*LsNa*	*LsnA*	*Lsna*	*lSNA*	*lSNa*	*lSnA*	*lSna*	*lsNA*	*lsNa*	*lsnA*	*lsna*
23270	*LSNA*	12	5	5	1	..	..	..	..	..	..	..	..	..	..	..	..
23293	"	18	5	..	..	..	..	..	..	5	1	..	..	..	..	..	..
23294	"	21	7	..	..	5	1	..	..	1	2	..	..	..	..	..	..
23295	"	17	..	11	..	8	..	2	..	..	..	..	..	..	..	..	..
23296	"	9	1	5	1	3	..	3	..	2	2	3	1	1	1	3	..
23298	"	27	12	..	..	..	..	..	..	..	..	..	..	..	..	..	..
23299	"	22	6	4	1	..	..	..	..	3	1	1	..	..	..	..	..
23301	"	14	2	5	3	..	..	..	..	3	..	1	..	..	..	..	..
23302	"	16	..	2	..	4	2	2	..	5	..	2	..	1	..	1	..
23303	"	13	7	..	..	..	..	..	..	4	2	..	..	..	..	..	..
23304	"	15	5	..	..	..	..	..	..	7	..	..	..	..	..	..	..
23307	"	25	4	5	..	..	..	..	..	..	..	..	..	..	..	..	..
23310	"	10	..	..	..	5	..	..	..	2	..	..	..	3	..	..	..
23313	"	10	3	9	3	4	1	..	..	3	1	1	..	1	..	1	..
23316	"	19	..	3	..	1	..	4	..	7	..	4	..	2	..	2	..
23318	"	24	..	6	..	..	..	..	..	7	..	3	..	..	..	..	..
23271	*LSNa*	..	20	..	..	..	..	..	..	..	4	..	..	..	..	..	..
23274	"	..	14	..	5	..	..	..	..	..	4	..	4	..	..	..	..
23275	"	..	13	..	2	..	3	..	2	1*	8	..	..	..	..	..	..
23276	"	..	34	..	..	..	7	..	..	..	..	..	..	..	..	..	..
23277	"	..	22	..	5	..	..	..	..	..	6	..	1	..	..	..	..
23279	"	..	29	..	..	..	9	..	..	..	..	..	..	..	..	..	..
23282	"	..	26	..	3	..	6	..	7	..	..	..	..	..	..	..	..
23285	"	..	20	..	3	..	1	..	3	..	7	..	2	..	2	..	1
23287	"	..	17	..	3	..	3	..	4	..	3	..	..	..	..	..	..
23288	"	..	31	..	..	..	..	..	..	..	11	..	..	..	..	..	..

TABLE 7 (continued)

CULTURE NUMBER	CHARACTER OF F_3 PARENT	F_3 DISTRIBUTION															
		LSNA	*LSNa*	*LSnA*	*LSna*	*LsNA*	*LsNa*	*LsnA*	*Lsna*	*lSNA*	*lSNa*	*lSnA*	*lSna*	*lsNA*	*lsNa*	*lsnA*	*lsna*
23290	*LSnA*	..	..	13	5	..	..	6	2	..	..	8	1	..	..	4	..
23309	"	..	..	9	..	..	..	1	..	..	..	3	..	..	..	..	..
23312	"	..	..	28	..	..	..	8	..	..	..	..	..	..	..	..	..
23317	"	..	..	29	..	..	..	7	..	..	..	..	..	..	..	..	..
23268	*LsNA*	..	..	..	..	11	1	5	3	..	..	..	..	5	..	1	2
23269	"	..	..	..	..	12	4	8	2	2*	..	..	..	4	1	2	..
23311	"	..	..	..	..	13	5	..	..	..	..	..	..	8	2	..	..
23273	*LsNa*	..	..	..	..	..	14	..	11	..	..	..	..	..	5	..	3
23283	"	..	..	..	..	..	21	..	6	..	..	..	..	..	..	..	..
23286	"	..	..	..	..	..	13	..	2	..	..	..	..	..	5	..	..
23291	*LsnA*	..	..	..	..	..	..	15	..	..	..	..	..	..	..	3	1
23292	"	..	..	..	..	..	..	14	1	..	..	..	..	..	..	7	..
23315	"	..	..	..	..	..	..	32	..	..	..	..	..	..	..	..	..
23278	*Lsna*	..	..	..	..	..	..	..	31	..	..	..	..	..	..	..	..
23280	"	..	..	..	..	..	..	..	22	..	..	..	..	..	..	..	5
23289	*lSNA*	..	..	..	..	..	..	..	..	13	7	4	3	4	2	..	..
23297	"	..	..	..	..	..	..	..	..	16	5	..	..	11	3	..	..
23300	"	..	..	..	..	..	..	..	..	26	6	..	..	..	..	..	..
23305	"	..	..	..	..	..	..	..	..	25	6	9	1	..	..	..	..
23314	"	..	..	..	..	..	..	..	..	32	..	5	..	..	..	..	..
23281	*lSNa*	..	..	..	..	..	..	..	..	..	16	..	2	..	2	..	9
23284	"	..	..	..	..	..	..	..	..	..	..	..	8	..	2	..	
23306	*lsNA*	..	..	..	..	..	..	..	..	..	..	..	..	21	6	6	3

*Probably result of natural crossing.

TABLE 8

CULTURE NUMBER	*SN*	*Sn*	*sN*	*sn*
23295	17	11	8	2
23296	14	10	5	6
23302	21	4	5	3
23313	17	13	6	1
23316	26	7	3	6
23275	21	2	3	2
23282	26	3	6	7
23285	27	5	3	4
23287	20	3	3	4
23289	20	7	6	0
23281	16	2	2	9

From measurement of ten individuals in each case, No. 154 had a mean length of 42.2 mm, while No. 025 had a mean length of 30.7 mm. The length of the first ten internodes in the F_1 was 37 mm. The distribution of density in F_2 is given in table 9. Some plants have been left out because they did not have good spikes for measurement of density.

As no extensive measurements have been made on the P_1 and F_2 populations, it is not possible to get a definite notion of the number of density factors concerned here. As to the relation of density with the other factors concerned in this cross, the inequality of the mean density values of the contrasting classes appears to be significant in the cases of *N versus n* and *S versus s*. If this correlation is due to a single factor in the *SN* linkage group, that factor should lie nearer to *S* than to *N*. In the absence of more extensive data, it is fruitless to speculate further.

THE INTERRELATIONS BETWEEN *N n*, *K k* AND *A* a^s a^j

HAYES and GARBER (1921) report the results of experiments conducted at the MINNESOTA AGRICULTURAL EXPERIMENT STATION, indicating the independent assortment between the factors affecting the contrasting characters:

(*K k*) Hooded *versus* awned,
(*B b*) Black *versus* white,
(*N n*) Hulled *versus* naked,
(a^s a^j) Two-rowed *versus* six-rowed.

The independent assortment between *Nn* and $a^s a^j$ has found confirmation in the foregoing cross. In crossing No. 038 ♀ of the genetic constitu-

tion, *NNkk*, by No. 100 ♂, of the genetic constitution, *nnKK*, the F_2 distribution is 166 *NK* : 64 *Nk* : 53 *nK* : 25 *nk*. In crossing No. 165 ♀, of the genetic constitution, a^fa^fKK, by No. 108 ♂, of the genetic constitution, *AAkk*, the F_2 distribution is 72 *AK* : 21 *Ak* : 22 a^fK : 12 a^fk. Simple inspection indicates the absence of significant correlation in the $Nk \times nK$ cross, while in the $Ak \times a^fK$ cross, the appearance of negative correlation needs no special comment except that data from actual cases of independent assortment may display with equal frequency apparent negative or positive correlation.

The F_2 results from crossing No. 173 ♀, of the genetic constitution $nnKKa^fa^f$, with No. 141 ♂ of the genetic constitution $NNkka^sa^s$, are as follows:

	22235	*23235*	*Total*	
NKa^s	46	63	109	
NKa^f	13	12	25	
Nka^s	10	7	17	
Nka^f	2	3	5	For independent assortment
nKa^s	9	18	27	
nKa^f	3	6	9	$\chi^2 = 19.42$; $P = 0.007$
nka^s	1	3	4	
nka^f	1	0	1	

The wide departure from the typical trihybrid distribution is primarily due to the great deviation from the 3 *K* : 1 *k* ratio (170 *K* : 27 *k*). Sixteen F_2 plants were carried to the F_3 generation. Two of these were awned and bred true. Of the 14 hooded plants, 6 bred true, and 8 segregated into hooded and awned with the summary ratio of 150 hooded : 38 awned. The F_3 tests thus indicate that these contrasting characters of the terminal appendage of the lemma are due to a single factor difference here, as has been found elsewhere. A great part of the discrepancy was probably due to the occurrence of selective elimination in the F_2 population grown in 1923, in which only about half of the seeds sown produced heads.

The discrepancy in the 3 *K* : 1 *k* ratio being primarily responsible for the large size of χ^2 in the above test, the typical trihybrid ratio is still good so far as the interrelations between the three pairs of factors are concerned. Thus, though the results from this cross can not be taken as a demonstration, it is, nevertheless, in conformity with the other experiments indicating independent assortment between *Nn*, *Kk* and Aa^sa^f.

TABLE 9

	LENGTH OF TEN BASAL INTERNODES IN MILLIMETERS																									MEAN IN MM	DIFFERENCE IN MEANS
	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53		
N	1	2	2	10	7	21	15	24	21	15	22	18	8	12	11	11	4	5	2	3	3	..	2	1	3	1	..	36.81±.22	2.01±.47
n	..	..	..	2	..	2	2	1	1	6	11	2	5	9	2	4	2	2	3	..	..	..	1	..	..	..	1	38.82±.42	
S	1	2	2	12	7	22	13	24	19	18	27	13	8	13	8	10	3	6	2	2	..	..	3	1	2	1	1	36.51±.22	3.07±.47
s	..	..	..	..	..	1	4	1	3	3	6	7	5	8	5	5	3	1	3	1	3	..	..	..	..	1	..	..	39.58±.41	
L	..	1	1	8	6	18	11	20	19	15	23	17	10	19	10	13	6	6	3	3	3	..	3	..	3	..	1	37.44±.22	1.24±.47
l	1	1	1	4	1	5	6	5	3	6	10	3	3	2	3	2	..	1	2	..	..	..	..	1	..	1	..	36.20±.42	
A	1	2	1	8	5	17	12	14	19	17	26	16	11	16	12	10	3	6	5	2	3	..	3	1	2	1	1	37.41±.22	.93±.40
a'	..	..	1	4	2	6	5	11	3	4	7	4	2	5	1	5	3	1	..	1	..	..	..	1	1	..	..	36.48±.33	
Total	1	2	2	12	7	23	17	25	22	21	33	20	13	21	13	15	6	7	5	3	3	0	3	1	3	1	1	37.09±.19	

CROSS INVOLVING *Bb*, *Ll*, $a^s a^f$

In crossing No. 127 ♀, of the genetic constitution $bbll a^f a^f$, with No. 171 ♂, of the genetic constitution $BBLLa^s a^s$, the F_2 distribution is as follows:

23 BLa^s
4 BLa^f
8 Bla^s
5 bLa^s
2 bLa^f
5 bla^s

With respect to the goodness of fit of this distribution to the typical trihybrid ratio, $\chi^2 = 8.48$ and $P = 0.295$. Thus, mathematically, the deviation is not excessive. But the distribution of 27 *BL* : 8 *Bl* : 7 *bL* : 5 *bl* suggests linkage. For the independent assortment between *Bb* and *Ll*, $\chi^2 = 1.90$ and $P = 0.595$. For linkage between *Bb* and *Ll* with the calculated crossover value of 39.40 ± 6.47, $\chi^2 = 0.93$. Thus, further evidence is needed for decision as to the existence of linkage.

The three F_3 populations in which segregation has taken place both with respect to *Bb* and *Ll* are given in table 10. All the three populations included in table 10 appear to exhibit the original linkage relation. The crossover value calculated from summary ratio of the three populations is 40.48 ± 4.72. The close agreement between the results of the F_2 and F_3 can be taken as a strong evidence for the assumed linkage relation.

TABLE 10

CULTURE NUMBER	*BL*	*Bl*	*bL*	*bl*
23263	18	5	2	2
23264	22	6	11	4
23266	15	1	4	1
Total	55	12	17	7

CROSS INVOLVING *Bb*, *Rr*, *Ll* AND *Ss*

The smooth-awn character of barley, in which the awn is smooth at its lower portion where the teeth are particularly strong in the common varieties, have been found by HARLAN (1920) to behave as a simple Mendelian recessive. The factor pair determining rough- and smooth-awned characters is here denoted by *Rr*. Strain No. 135 ♀, of the genetic

TABLE 11

CULTURE NUMBER	CHARACTER OF F_2 PARENT	F_3 DISTRIBUTION															
		BRLS	*BRLs*	*BRlS*	*BRls*	*BrLS*	*BrLs*	*BrlS*	*Brls*	*bRLS*	*bRLs*	*bRlS*	*bRls*	*brLS*	*brLs*	*brlS*	*brls*
23476	*BRLS*	15	..	6	..	3	..	2	..	..	..	..	..	..	..	..	..
23477	"	9	4	4	1	1	..	..	..	5	..	..	..	1	..	..	..
23479	"	18	3	5	1	2	2	1	..	..	..	..	..	..	..	..	..
23480	"	21	4	..	..	..	..	..	..	6	2	..	..	..	..	..	..
23482	"	8	4	2	1	1	2	1	1	5	4	..	..	1	..	1	..
23486	"	5	4	4	..	5	..	..	..	1	1	..	..	3	..	..	..
23491	"	8	4	..	..	7	2	..	..	12	..	..	..	1	1	..	..
23493	"	11	2	..	..	2	2	..	..	1	..	..	..	..	1	..	..
23500	"	13	..	1	..	7	..	..	..	2	..	2	..	4	..	1	..
23506	"	14	4	4	..	2	1	1	2	2	5	..	..	..	..	..	..
23510	"	11	4	9	1	..	..	..	..	..	..	..	..	..	..	..	..
23517	"	10	..	4	..	1	..	..	..	2	..	1	..	2	..	..	..
23518	"	18	4	4	2	..	..	..	..	..	..	..	..	..	..	..	..
23526	"	5	..	2	..	1	1	..	..	2	..	3	..	..	..	..	..
23530	"	9	..	2	..	3	..	1	..	3	..	2	..	..	..	..	..
23536	"	12	2	5	..	4	..	..	2	6	..	1	..	..	1	..	1
23538	"	4	2	4	..	2	4	1	1	2	..	2	..	2	..	..	..
23539	"	9	4	3	..	7	2	2	1	..	..	..	..	..	..	..	..
23540	"	21	7	8	1	..	..	..	..	..	..	..	..	..	..	..	..
23492	*BRLs*	..	16	..	6	..	..	..	..	..	6	..	2	..	..	..	..
23508	"	..	13	..	9	..	..	..	..	..	..	..	..	..	..	..	..
23528	"	..	17	..	..	..	6	..	..	..	9	..	..	..	..	..	..
23581	"	..	4	..	6	..	4	..	..	..	3	..	3	..	..	..	..

TABLE 11 (continued)

CULTURE NUMBER	CHARACTER OF F_3 PARENT	F_3 DISTRIBUTION														
		BRLS	*BRLs*	*BRlS*	*BRls*	*BrLS*	*BrLs*	*BrlS*	*Brls*	*bRLS*	*bRLs*	*bRlS*	*bRls*	*brLS*	*brLs*	*brlS*	*brls*
23484	*BRlS*	..	..	24	13	..	..	..	..	..	..	..	..	..	..	..	..
23489	"	..	..	19	..	..	..	..	..	..	..	9	..	..	..	..	..
23502	"	..	..	15	..	..	..	..	..	..	..	6	..	..	..	..	..
23505	"	..	..	10	8	..	..	..	..	..	..	6	2	..	..	..	..
23515	"	..	..	18	3	..	..	1	4	..	..	7	2	..	..	..	..
23516	"	..	..	27	6	..	..	..	..	..	..	..	..	..	..	..	..
23522	"	..	..	19	4	..	..	..	..	..	..	8	1	..	..	..	..
23525	*BRlS*	..	..	12	..	..	..	2	..	..	..	2	..	..	..	2	..
23527	"	..	..	22	5	..	..	..	..	..	..	1	..	..	..	..	..
23532	"	..	..	20	..	..	..	..	..	..	..	5	..	..	..	..	..
23533	"	..	..	7	..	..	..	..	..	..	..	..	..	..	..	..	..
23483	*BRls*	..	..	..	17	..	..	..	3	..	..	..	4	..	..	..	2
23487	"	..	..	..	19	..	..	..	..	..	..	..	11	..	..	..	..
23496	"	..	..	..	27	..	..	..	..	..	..	..	12	..	..	..	..
23481	*PrLS*	..	..	..	..	12	9	..	..	..	..	..	..	2	3	..	..
23501	"	..	..	..	..	5	5	2	3	..	..	..	..	4	3	..	1
23509	"	..	..	..	..	10	..	2	..	..	..	..	..	5	..	..	..
23511	"	..	..	..	..	14	7	..	..	..	..	..	..	9	3	..	..
23520	"	..	..	..	..	21	11	..	..	..	..	..	..	..	..	..	..
23521	"	..	..	..	..	6	4	..	..	..	..	..	..	..	..	..	..
23524	"	..	..	..	..	12	2	5	2	..	..	..	..	6	2	1	1
23529	"	..	..	..	..	15	..	6	..	..	..	..	..	..	..	..	..
23537	"	..	..	..	..	10	5	..	..	..	..	..	..	..	1	..	..

TABLE 11 (continued)

CULTURE NUMBER	CHARACTER OF F_3 PARENT	F_3 DISTRIBUTION															
		BRLS	*BRLs*	*BRlS*	*BRls*	*BrLS*	*BrLs*	*BrlS*	*Brls*	*bRLS*	*bRLs*	*bRlS*	*bRls*	*brLS*	*brLs*	*brlS*	*brls*
23478	*BrLs*	..	..	..	..	..	19	..	10	..	..	..	..	..	..	..	..
23490	"	..	..	..	..	..	19	..	..	..	..	..	..	..	4	..	..
23485	*bRLS*	..	..	..	..	..	..	..	..	4	2	8	1	4	3	..	..
23495	"	..	..	..	..	..	..	..	..	14	..	7	..	5	..	3	..
23497	"	..	..	..	..	..	..	..	..	13	..	2	..	..	..	..	..
23503	"	..	..	..	..	..	..	..	..	18	..	9	..	8	..	2	..
23512	"	..	..	..	..	..	..	..	..	18	3	9	1	..	..	..	..
23513	"	..	..	..	..	..	..	..	..	11	..	3	..	4	..	..	..
23534	"	..	..	..	..	..	..	..	..	13	2	3	1	..	..	..	..
23507	*bRLs*	..	..	..	..	..	..	..	..	..	17	..	4	..	9	..	..
23504	"	..	..	..	..	..	..	..	..	..	16	..	2	..	8	..	..
23488	*bRlS*	..	..	..	..	..	..	..	..	..	..	18	1	..	..	5	7
23494	"	..	..	..	..	..	..	..	..	..	..	22	..	..	..	..	..
23498	"	..	..	..	..	..	..	..	..	..	..	21	6	..	..	5	1
23499	"	..	..	..	..	..	..	..	..	..	..	17	4	..	..	4	1
23519	"	..	..	..	..	..	..	..	..	..	..	19	10	..	..	..	..
23523	"	..	..	..	..	..	..	..	..	..	..	17	4	..	..	2	6
23535	*bRls*	..	..	..	..	..	..	..	..	..	..	..	18	..	..	..	8

constitution *bbllRRSS*, was crossed with No. 174 ♂, of the genetic constitution *BBLLrrss*. The F_2 distribution is as follows:

28 *BRLS*	10 *bRLS*
8 *BRLs*	2 *bRLs*
15 *BRlS*	8 *bRlS*
4 *BRls*	1 *bRls*
15 *BrLS*	2 *brLS*
5 *BrLs*	1 *brLs*

Thus, no class with *rl* is present and the relation between *Rr* and *Ll* is represented by the distribution 48 *RL* : 28 *Rl* : 23 *rL* : 0 *rl*. If no other evidence were available, this distribution would favor the notion that a single factor is responsible for both the dominant rough-awn character and the recessive short-hair character, and its allelomorph is responsible for both the recessive smooth-awn character and the dominant long-hair character. That this is not the case will be seen from the F_3 results.

The distribution with respect to *Bb* and *Ll* is 56 *BL* : 19 *Bl* : 15 *bL* : 9 *bl*. Assuming that linkage exists between *Bb* and *Ll*, as indicated by the previous cross, the crossover value is 44.04 ± 4.74 percent. For the calculated linkage relation, $\chi^2 = 0.70$; for independent assortment, $\chi^2 = 1.97$.

The relation between *Bb* and *Rr* is represented by the distribution 55 *BR* : 20 *Br* : 21 *bR* : 3 *br*. The crossover value calculated from this distribution is 41.48 ± 5.45 percent. The χ^2 values for the calculated linkage relation and for independent assortment are 0.42 and 2.09, respectively.

Table 11 contains the results from 64 F_3 populations grown from the F_2 plants of this cross.

The F_3 populations which are the progenies of the F_2 plants heterozygous for both *R* and *L* are assembled in table 12. The F_3 results leave no doubt that *Rr* and *Ll* are entirely different factor pairs. In fact, the repulsion has been very much weakened. The crossover value calculated from the summary of the thirteen populations showing repulsion (158 *RL* : 63 *Rl* : 88 *rL* : 9 *rl*) is 28.70 ± 3.43 percent. The summary of the seven populations showing coupling (132 *RL* : 34 *Rl* : 24 *rL* : 13 *rl*) gives a crossing over value of 34.54 ± 2.89 percent. Whether the great discrepancy between the linkage intensities exhibited by the F_2 and F_3 populations is primarily due to environmental influence or to recombination of genetic factors that modify the frequency of crossing over is difficult to ascertain. Whatever little evidence we have is in favor of the latter explanation; for the close agreement of the two crossover values between *Bb* and *Ll* obtained from the F_2 and F_3 data of the cross No. 127 ×

No. 171 indicates the probable ineffectiveness of ordinary environmental changes in the field in inducing great variations in the frequency of crossing over in barley, while the great variations in linkage intensities exhibited by the different F_3 populations, which will be evident when the other relations have been considered, are best explained by the recombination of genetic factors modifying the frequency of crossing over.

TABLE 12

CULTURE NUMBER	*RL*	*Rl*	*rL*	*rl*
23476	15	6	3	2
23477	18	5	2	0
23479	21	6	4	1
23482	21	3	4	3
23506	25	4	3	3
23530	12	4	3	1
23536	20	6	5	3
Total coupling	132	34	24	13
23486	11	4	8	0
23500	15	3	11	1
23517	12	5	3	0
23526	7	4	2	0
23538	8	6	8	0
23539	13	3	9	3
23531	10	4	6	0
23485	6	9	7	0
23495	14	7	5	3
23503	18	9	8	2
23513	11	3	4	0
23507	17	4	9	0
23504	16	2	8	0
Total repulsion	158	63	88	9

The F_3 populations grown from the F_2 plants heterozygous for both *B* and *R* are assembled in table 13. The summary proportion for all the populations showing the original linkage relation (144 *BR* : 55 *Br* : 73 *bR* : 12 *br*) gives a crossover value of 31.40 ± 3.56 percent. The summary proportion for all the populations showing the reversed linkage (101 *BR* : 23 *Br* : 21 *bR* : 16 *br*) gives a crossover value of 32.26 ± 6.61 percent. The close agreement between the two values argues for the correctness of the classification as to the real genetic constitution of the F_2 parents. The degree of repulsion has been considerably increased in comparison with the F_2 data.

TABLE 13

CULTURE NUMBER	*BR*	*Br*	*bR*	*br*
Coupling				
23477	18	1	5	1
23486	13	5	2	3
23493	13	4	1	1
23500	14	7	4	5
23517	14	1	3	2
23525	12	2	2	2
23484	17	3	4	2
Total coupling	101	23	21	16
Repulsion				
23482	15	5	9	2
23491	12	9	12	2
23506	22	6	7	0
23526	7	2	5	0
23530	11	4	5	0
23536	19	6	7	2
23538	10	8	4	2
23528	17	6	9	3
23531	10	4	6	0
23515	21	5	9	1
Total repulsion	144	55	73	12

TABLE 14

CULTURE NUMBER	*BL*	*Bl*	*bL*	*bl*
23477	14	5	6	0
23482	15	5	10	1
23486	14	4	5	0
23500	20	1	6	3
23506	21	7	7	0
23517	11	4	4	1
23526	7	2	2	3
23530	12	3	3	2
23536	18	7	7	2
23538	12	6	4	2
23492	16	6	6	2
23531	8	6	3	3
23501	10	5	7	1
23509	10	2	6	0
23524	14	7	8	2
Total	202	70	84	22

The F_3 populations grown from the F_2 plants heterozygous for both *B* and *L* are assembled in table 14. If all these populations are summed up, the proportion is 202 *BL* : 70 *Bl* : 84 *bL* : 22 *bl*, which is in the form of repulsion instead of coupling. This is, however, not unexpected, as, besides the low intensity of linkage and the considerable size of its probable error, populations with the reversed linkage relation may be present in excess of their normal proportion as a consequence of ordinary fluctuation.[4] No attempt is made to classify the populations in this case, as too much uncertainty is involved here.

CONCLUSION REGARDING THE *BRL* GROUP

Considering all the evidence that has been presented, it is certain that *Bb*, *Rr*, and *Ll* lie in the same linkage group. The most probable sequence is *BRL*. The distance between *R* and *L* is probably considerably shorter than that between *B* and *R*, though on account of the great differences in crossover values between F_2 and F_3 results, the assignment of definite distances must await more extensive experiments.

In this connection it may be noted that COLLIN and TROUARD-RIOLLE (1923) claim to have found dissociation of characters in F_1 resulting from crossing a black smooth-awned barley with a white rough-awned barley. The following is quoted verbatim from their original paper.

"Les types les plus curieux, en F_1, sont épis dont les grains restent noirs, mais d'un noir très attenué, tandis que les barbes sont tout blanches et parfaitment lisses; la form lisses à grain blancs recherchée par l'agriculture, fait complètement défaut en F_1 et n'apparaita qu'en F_2 dans la descendance des épis noir rugueux. . . . . Tous les grains provenant d'épis lisses, que soit la teint des barbes, ont donné en F_2 des épis lisses, gris ou noir, comme si la dissociation était accomplié des F_1."

The rough-awned forms are reported to segregate in F_2 into all the nine possible combinations of black, gray and white color, with rough, smooth, and smooth-rough awn, but no numerical data are given.

It is interesting to note that all the F_1 smooth-awned spikes are reported to have black or gray kernels and give no white spikes in F_2. It seems that these investigators did not appreciate the developmental differences in the characters under consideration, when they attach genetic significance to

[4] Denoting the amount of crossing over by *c*, the coefficient of the F_2 gametes with recombined linked factors is $\frac{c-c^2}{1-2c-2c^2}$, which is intermediate between *c* and 0.5, the value for independent assortment, (between $1-c$ and 0.5 if *c* ever exceeds 0.5). The value expressed by this formula approximates the value calculated from the summary F_3 distribution when a large number of equal-sized F_3 populations are dealt with.

the differences among spikes of the same plant. As the progenies of all the "rough" spikes are lumped together in an ambiguous manner, I seriously doubt whether their "rough" spikes of plants bearing other smooth spikes were really rough. I believe that the supposed F_1 segregants merely represent failures in the intended pollinations, which were probably due to faulty technique, allowing self-pollination to occur (the occurrence of parthenogenesis has never been reported in barley and therefore can scarcely be considered an available interpretation).

THE FACTORS FOR GLUME WIDTH AND THEIR RELATIONS

In crossing No. 140 ♀ with No. 136♂, both of which possess the normal narrow outer glumes, the F_1 plants are like the parental forms with respect to this character. In the F_2, forms appeared in which the two outermost glumes (or sometimes the two central outer glumes) are widened and with awns more elongated. The proportion is 91 narrow : 21 wide, which is in perfect agreement with the 13 : 3 ratio. Evidently one of the parents must carry both the dominant factor for wide and the dominant factor for inhibition of the same character, while the other parent carries both of the recessives.

Besides this hidden difference, the two parents differ in two other obvious respects, 140 being of the *LLa^{s}a^s* type and 136 of the *lla^{j}a^j* type. The F_2 distribution with respect to all these characters is given below:

Narrow	*La^s*	54	
Narrow	*La^j*	13	
Narrow	*la^s*	17	
Narrow	*la^j*	7	For independent assortment,
Wide	*La^s_j*	14	$\chi^2 = 3.35$; $P = 0.848$
Wide	*La*	3	
Wide	*la^s*	2	
Wide	*la^j*	2	
Total		112	

Twelve F_3 populations were grown and examined for the character of the outer glume. The results are given in table 15. As a whole these F_3 results conform with the expectation. If more wide F_2 plants had been grown, we would find two-thirds of them exhibiting the 3 wide : 1 narrow ratio in F_3.

TABLE 15

NUMBER OF CULTURE	CHARACTER OF F_2 PARENT	F_3 DISTRIBUTION	
		Narrow	Wide
23419	Narrow	15	1
23420	Narrow	13	3
23421	Narrow	20	0
23422	Narrow	25	0
23423	Wide	0	32
23424	Narrow	25	7
23425	Narrow	37	0
23426	Wide	0	34
23427	Narrow	31	0
23428	Narrow	37	5
23429	Narrow	25	0
23430	Narrow	29	6

In crossing No. 125 ♀, which was short-haired and narrow, with No. 213♂, which was long-haired and wide, the F_1 is long-haired and narrow and the F_2 distribution is as follows:

Narrow *L*,	169
Narrow *l*,	52
Wide *L*,	53
Wide *l*,	12
Total	286

Though the outermost glumes of No. 213 do not exceed those of the normal forms conspicuously in width, many F_2 plants with conspicuously wide outer glumes are found in this cross as in the previous cross. Evidently one or more modifying factors affecting the width are at work, but their exact nature has not been determined.

Numbers 125 and 136 are from the same origin and probably identical strains. Hence, it may be assumed that they both have the genetic constitution *IIWW*, *W* standing for wide, and *I*, for inhibitor of wide.

Thus it appears that:

No. 213 has the constitution *iiWW;*

No. 140 has the constitution *iiww*.

TSCHERMAK (1919) mentions the wide glume character as a simple Mendelian recessive. Probably he has dealt with the factor pair *Ii*. SCHIEMANN (1921) reports the occurrence of wide plants in the F_2 of a cross between two narrow forms and discusses the origin and variability of the anomaly at some length. The proportion of wide plants which she

secured in the F_2 was too small to fit in the 13 : 3 scheme. As about 50 percent of her F_2 plants failed to ripen, due to segregation in rate of growth and in winter hardiness, there may have been selective elimination of the wide forms on account of linkage with some of the growth factors.

A variety of *H. vulgare*, No. 203, has all the outer glumes glabrous, very wide, and possessing awns as long and coarse as those of the lemmas. To distinguish it from No. 213, which has only slightly widened outermost glumes with short fine awns, this strain is called "extended."

In crossing No. 136 ♀ (*IIWWee*) with No. 203♂ (*EE*), the monohybrid distribution, 54 extended : 117 intermediate : 59 normal, has been secured in F_2. Evidently *I* does not inhibit the effect of *E* as it inhibits the effect of *W*. The relation between *E* and *W* has not been determined, as the attempt to cross No. 203 with No. 213 was not successful.

The cross between No. 151 ♀ (*eeBBa^{s}a$_s$*) and No. 203♂ (*EEbba^{f}a^f*) gives the following F_2 distribution.

BEa^s	84	
BEa^f	26	
Bea^s	35	
Bea^f	7	For independent assortment,
bEa^s	25	$\chi^2 = 5.24$; P = 0.631
bEa^f	12	
bea^s	11	
bea^f	1	
Total	201	

Though the distribution agrees well with expectation in case of a typical trihybrid, the proportion 109 *Ea^s* : 38 *Ea^f* : 46 *ea^s* : 8 *ea^f* suggests the existence of linkage. The calculated crossover value is 40.52 ± 3.93 percent. Besides, the distribution 119 *Ba^s* : 33 *Ba^f* : 36 *ba^s* : 13 *ba^f* gives a crossover value of 44.02 ± 3.32 percent; the distribution 110 *BE* : 42 *Be* : 37 *bE* : 12 *be* gives a crossover value of 46.26 ± 3.71 percent. In each of the three cases, χ^2 for the calculated linkage intensity is less than 1.

It is probable that linkage actually exists between *Ee* and *a^{s}a^f*, as the calculated crossover value differs from fifty percent by 2.4 times the probable error of the former. χ^2 for independent assortment between *Ee* and *a^{s}a^f* is 3.64, setting the value of P at 0.309. While this deviation can not be considered as significant by itself, the probability of linkage is increased by the fact that χ^2 for the assumed linkage intensity is less than 1.

The evidence presented in previous sections concerning linkage between *Bb* and *Ll* and independent assortment between *Ll* and *Aa^{s}a^f* is against

the assumption that all the three factors involved in this cross lie in the same linkage group. Moreover, the calculated linkage intensities between *Bb* and $a^{s}a^{f}$ and between *Bb* and *Ee* are not significant in comparison with their probable errors, and χ^2 for independent assortment in each of these cases is small.

If the three factors really belong to the same linkage group with the sequence *BEA*, then it must be assumed, if the theory of linear arrangement of genes is applicable here, that *B* and *A* are very far apart from each other and that the direct crossover value of 46. 26 percent represents a distance probably exceeding one-hundred units, as a considerable amount of double crossing over may be expected between *B* and *E* and between *E* and *A*. Similar assumption must then also be made for a previous cross (No. 127 × No. 171) involving *L*, *B* and *A*, where the sequence for the hypothetical group is *LBA* and the distance between *L* and *A* must be extremely long. This consideration is further indication that the *BEA* group (whose establishment would lead to the establishment of the *LRBEA* group) is highly improbable.

CROSS INVOLVING Aa^{f} AND *Mm*

A new variety of *H. vulgare* with branching inflorescence and greatly increased number of nodes has been described by the writer (HOR 1922). This character has been determined by HARLAN and POPE (1922) to behave as a simple Mendelian recessive. No. 156 ♀, a variety of *H. deficiens* with normal growth form (*AAMM*), was crossed to No. 205♂, the six-rowed, many-noded, branching barley ($a^{f}a^{f}mm$). The F_2 distribution is as follows, 123 *AM* : 43 *Am* : 39 $a^{f}M$: 15 $a^{f}m$.

Independent assortment is indicated, as this is a close fit to the typical dihybrid distribution, χ^2 being less than 1.

DISCUSSION

UBISCH (1916) has found the factor concerned in fertility,[5] *Z*, to be linked with *G*, one of the factors concerned in the barbing of the inner pair of nerves of the lemma, with a gametic ratio of "5 : 1." He also found (UBISCH 1919, 1921) *L* (main factor concerned in density), *A* (main factor for awn length), and *S* (factor for hulllessness) to lie in the same linkage group. The crossover values[6] which he has obtained for *LA*, *AS* and *LS*

[5] As no uniform symbols have been adopted, the original symbols as used by the different investigators are given with the original definitions.

[6] These values have been calculated by UBISCH from the nearest gametic ratio of *n* : 1, where *n* is an integer, and thus are not exact.

are 20 percent, 14.3 percent and 16.7 percent, respectively. He mentions the inapplicability of the theory of the linear arrangement of genes in this case; but since these values have not been derived from the same cross, it is probable that the discrepancy is due to different genetic factors modifying the frequency of crossing over, if the identities of his *L*, *S* and *A* in the different crosses are granted.

MIYAKE and IMAI (1922) report the establishment of two linkage groups, which are given below:

Group I

Nn, hulled *versus* naked,
L$_1$*l*$_1$, *L*$_2$*l*$_2$, } concerned in density,
A$_1$*a*$_1$, one of the factor pairs concerned in awn length,
C$_1$*c*$_1$, one of the factors concerned in hooded *versus* awned.

Group II

Uu, tall and slender *versus* short and stout,
A$_2$*a*$_2$, one of the factor pairs concerned in awn length,
Ii, awnless *versus* awned lateral floret,
Dd, two-rowed *versus* six-rowed,
P$_1$*p*$_1$, one of the factor pairs concerned in purple *versus* colorless kernel.

It does not seem to the writer that these two groups have been established beyond doubt with respect to all the factors therein included. The existence of *L*$_2$ as distinct from *L*$_1$ has been inferred by these investigators from the difference of 10 percent in the value of crossing over with *N*. Since the crossover values have been obtained from different crosses there is the possibility here too that the difference is due to different genetic factors affecting the frequency of crossing over. As these investigators report no measurements, it is difficult to appreciate the identities of the two density and four awn-length factors (two of which are not included in either of the two groups) assumed by them. *Dd* and *Ii*, which they have put in the same linkage group, evidently represent the two effects of the $a^s a^f$ pair of the $Aa^s a^f$ series discussed in this paper.

The group I of MIYAKE and IMAI is the same as the *LSA* group of UBISCH and the *NS* group determined in this study.

The group II of MIYAKE and IMAI is the same as the *ZG* group of UBISCH and the probable group *EA* in this study.

The group *BRL* determined in this study is probably independent of either of the above two groups.

Whether the linkage between white No. 3 and *chlorina* forms of barley found by NILSSON-EHLE (1922) constitutes an additional group or belongs to one of the three above groups is not known.

SUMMARY

Ten factor pairs and one triple-allelomorph series have been dealt with, and evidence has been presented for linkage or independent assortment among them.

Two linkage groups have been definitely established in this study. One of these groups includes the factors concerned in the color of the kernel, the texture of the awn, and the length of hair on the rachilla. The other group includes the factors concerned in the adherence of the lemma to the caryopsis and the extent of pubescence on the outer glume. Linkage probably exists between the factors concerned in the fertility of the lateral floret and the extension of the outer glume, which constitute a separate group from the other two.

A study of the distribution of the density of the spike in an F_2 population indicates the probability of one or more density factors lying in the second of the above-mentioned groups, which is in agreement with the findings in previous works.

The linkage groups of barley which have thus far been determined by different investigators have been brought together in the discussion.

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INHERITANCE IN *NICOTIANA TABACUM*. IV. THE TRISOMIC CHARACTER, "ENLARGED"

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INTRODUCTION

In our studies of inheritance in *Nicotiana Tabacum*, we have discovered a character, "enlarged," which does not depend upon Mendelian genic differences; but which is due to the presence of an extra chromosome. It marks the beginning of a study of abnormal chromosomal constitution in this species. So much interest has been aroused of late in the effects of abnormal chromosome constitution, particularly as a result of the important work of BLAKESLEE (1922) and his associates, that we propose to follow out our particular example in all its ramifications. Since this project will require time for its completion, we have decided to publish a preliminary report of our investigations to date. The results thus far secured establish adequately the mode of transmission of enlarged when contrasted with normal. Cytological confirmation of our conclusions has been secured by Miss MILDRED HOLLIS, who will publish her results independently. Further work on the relation of enlarged to a variety of Mendelian contrasts is in progress.

DESCRIPTION OF ENLARGED

Enlarged is characterized by an increase in flower size, readily determined by taking measurements of length and spread of corolla. The

length of corolla in enlarged plants is approximately 8 mm greater than in normal plants of the same population; the spread is increased about 5 mm, but the differences depend somewhat upon the particular population. Length is most useful as a diagnostic feature, because the difference between the two types is greater and apparently less subject to variation than spread. Correlation tables of length and spread of corolla in populations segregating for enlarged and normal, typically exhibit a distinct separation of the population into two groups as shown in table 1, where the normal group occupies the upper left-hand and enlarged the lower right-hand portion of the table. In our work with enlarged, we measure both length and spread of corolla and construct correlation tables for each population, but in this paper distributions for length only are given in order to reduce tabular material to a minimum.

TABLE 1

Correlation table of length with spread of corolla in 22.083, the progeny of a self-fertilized enlarged plant. The upper left group is the normal and the lower right the enlarged class.

Length in millimeters	Spread in millimeters										Total
	31	32	33	34	35	36	37	38	39	40	
46				1	1						2
47	1	3	2	3	1						10
48			4	3	2						9
49		1	1	1	2	1					6
50											—
51											—
52											—
53											—
54											—
55						1	1	4	1	1	8
56						2	1	3	4		10
57								1	1	1	3
58										1	1
59								1			1
Total	1	4	7	8	6	4	2	9	6	3	50

Aside from the increase in flower size there appear to be no important differences between normal and enlarged plants. It has always been more difficult to secure seed from enlarged plants, because the flowers are abscissed more readily, particularly when enclosed in paraffin bags. As a consequence, perhaps, of the freer setting of seed, normal plants are usually somewhat less robust than enlarged plants in the same populations. There are minor differences in appearance of the flowers of the two types, which, in conjunction with the size differences, make it possible for one familiar

with the material to classify them with a fair degree of accuracy without resorting to measurement.

THE ORIGIN OF ENLARGED

The enlarged type first appeared in a study of derivatives of a cross between the *Tabacum* varieties, *angustifolia* and *macrophylla* (cf. SETCHELL, GOODSPEED and CLAUSEN 1922). One of the derivatives of this hybrid was a form which we called *auriculata*, because it had a leaf, the blade of which was sharply constricted at the base, almost to the midrib. In F_5 this derivative had apparently become constant. In order to study the relation of its constricted leaf-base type to the broad base type of *macrophylla*, reciprocal crosses were made between the two (SETCHELL, GOODSPEED and CLAUSEN 1922, p. 488). In 1915 four F_1 populations of 50 plants each were grown, a total of 200 plants. The populations were uniform save for one plant, $15F_1H_{55}P_{16}$, which produced distinctly larger flowers than the other plants, although it appeared to be identical with them in other respects. This one plant is the source of our "enlarged" lines.

That the original enlarged F_1 plant was not an experimental error was clearly established by the parallel behavior of its F_2 progeny with those from normal F_1 plants. The *auriculata* parent had pink flowers and constricted leaf base contrasted with red flowers and broad leaf base of *macrophylla*. The F_1 plants, including $15F_1H_{55}P_{16}$, were pink broad, and the F_2 progenies were distributed in the expected four classes in the ratio, 9 pink broad : 3 pink constricted : 3 red broad : 1 red constricted (cf. SETCHELL, GOODSPEED and CLAUSEN 1922, p. 489).

THE NORMAL BEHAVIOR OF *auriculata* × *macrophylla*

In order to serve as a means of comparison with behavior in enlarged lines we have grown a number of populations of plants in order to determine the normal behavior in crosses between the *auriculata* derivative and *macrophylla*. Flower measurements were not taken in the original F_1 populations, because these differences were not considered important at the time. Consequently, when we had determined that the enlarged flower size of $15F_1H_{55}P_{16}$ represented a real genetic difference, as shown by the distributions for flower length in its progeny, we grew another F_1 population from the same seed packet which had produced the enlarged plant. The distribution for length in this progeny is shown in table 2 under the caption $17F_1H_{55}$. The distribution is obviously of the normal type.

The distributions of six F_2 populations from normal F_1 plants are also contained in this table. All of these, likewise, are of the normal type. They exhibit an increase in the standard deviation of the kind we have learned to expect in F_2 for crosses involving differences in flower measurements. The different F_2's exhibit slight differences in means, which might be held to indicate that the F_1 population was not genetically homogeneous, possibly because the *auriculata* derivative had not yet become completely homozygous. The differences, however, are of doubtful genetic significance, because it was not possible to take all these measurements under the same conditions or to have the same person make them. Thus $16F_2H_{55}P_{29}$ and $17F_2H_{55}P_{29}$ represent two F_2 populations grown in different years from the same packet of seed, yet they exhibit a difference of 2.46 ± 0.16 mm in mean length.

The last two populations in the table are F_3 progenies. They differ 3.05 ± 0.11 mm in mean length. They exhibit slight decreases in the standard deviation as compared with F_2 values. The last population contained two plants which had a very much shorter tube length than the sister plants. We have found that these extremely short flowers are produced by stunted plants, and that they have no genetic significance. They are accordingly omitted in the computation of statistical constants in this and other distributions in which they appear. We may conclude from the results of this series of populations that segregation occurs for minor flower-size differences in the *auriculata-macrophylla* series aside from the existence of the enlarged condition. This fact must be considered in the interpretation of behavior in the enlarged lines, as will be evident when we come to a discussion of them.

THE BEHAVIOR OF NORMAL SEGREGANTS FROM ENLARGED

We have collected in table 3 the distributions which pertain to normal segregants of "enlarged" plants. The two F_3 populations are the progeny of normal segregants of the original enlarged plant, $15F_1H_{55}P_{16}$. They were selected from the lower portion of the range of $16F_2H_{55}P_{16}$, as may be seen by reference to table 4. These two populations obviously differ significantly in mean tube length, but they are both normal frequency distributions. From each of these two populations two selections were made from which the F_4 populations, $18F_4H_{55}P_{16}P_{39}P_{10}$, $18F_4H_{55}P_{16}P_{39}P_{44}$, $18F_4H_{55}P_{16}P_{45}P_{72}$ and $18F_4H_{55}P_{16}P_{45}P_{82}$, were grown. These four populations demonstrate clearly that size differences, aside from those characteristic of enlarged, may be established in the normal lines of the *auriculata-macrophylla* cross.

TABLE 2

Distributions for tube length in the progeny of normal plants of auriculata×macrophylla.

GARDEN NUMBERS	TUBE LENGTH IN MILLIMETERS 39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	TOTAL	MEAN	S. D.
$17F_1H_{55}$	..	..	..	..	..	..	1	1	4	49	35	5	..	..	..	..	95	48.38±.05	0.78±.04
$16F_2H_{54}P_7$	..	..	2	7	12	10	8	6	5	..	..	..	..	..	..	..	50	44.06±.16	1.64±.11
$16F_2H_{55}P_{29}$	..	..	..	2	12	7	18	5	5	..	..	..	..	..	..	..	49	44.51±.13	1.34±.09
$17F_2H_{55}P_{29}$	..	..	..	..	2	1	12	15	35	22	11	1	..	..	..	..	99	46.97±.09	1.34±.06
$18F_2H_{55}P_{40}$	..	..	..	..	..	..	1	1	12	20	34	23	4	1	2	..	98	48.91±.09	1.33±.06
$18F_2H_{55}P_{41}$	..	..	..	..	1	..	1	2	7	15	26	22	19	2	1	..	96	49.26±.11	1.58±.08
$18F_2H_{55}P_{46}$	..	..	..	..	..	2	..	5	1	13	31	22	11	5	..	1	91	49.26±.12	1.63±.08
$18F_2H_{55}P_{29}P_{67}$	..	..	..	..	..	..	..	1	3	13	31	34	11	4	1	..	98	49.51±.08	1.19±.06
$18F_2H_{55}P_{29}P_{82}$	1*	1*	..	..	..	5	17	26	30	15	4	..	..	..	..	..	99	46.46±.08	1.21±.06

*Not included in computations of statistical constants.

TABLE 3

Distribution of tube lengths of progenies of normal segregants from enlarged.

GARDEN NUMBERS	PARENTAL VALUES MM	TUBE LENGTH IN MILLIMETERS 35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	TOTAL	MEAN	S. D.
$17F_3H_{55}P_{16}P_{39}$	43	..	..	..	1	3	6	19	30	24	11	3	1	..	1	..	..	..	..	99	42.21±.10	1.52±.07
$17F_3H_{55}P_{16}P_{45}$	40	..	..	..	..	..	..	..	..	3	9	14	23	22	14	3	1	..	..	89	46.25±.11	1.47±.07
$18F_4H_{55}P_{16}P_{12}$	?	..	..	..	..	..	..	..	2	1	11	15	13	6	..	..	..	..	..	48	45.12±.11	1.14±.08
$18F_4H_{55}P_{16}P_{30}$	44	..	..	..	..	..	..	1	3	12	41	33	8	2	..	..	..	..	..	100	44.34±.07	1.02±.05
$18F_4H_{55}P_{16}P_{11}P_{39}$	46	..	..	..	..	..	3	7	12	13	20	28	13	..	..	..	..	..	1*	97	43.83±.11	1.61±.08
$18F_4H_{55}P_{16}P_{21}P_{31}$	44	..	..	..	..	..	..	..	..	1	4	55	38	2	..	..	..	..	..	100	45.36±.04	0.64±.04
$18F_4H_{55}P_{16}P_{39}P_{10}$	43	..	..	..	7	31	43	18	1	..	..	..	..	..	..	..	..	..	..	100	39.75±.06	0.86±.04
$18F_4H_{55}P_{16}P_{39}P_{44}$	39	1*	..	..	6	38	42	11	1	..	..	..	..	..	..	..	..	..	..	99	39.62±.06	0.80±.04
$18F_4H_{55}P_{16}P_{45}P_{72}$	43	..	..	..	..	..	1	5	8	27	39	14	2	1	..	..	..	..	..	97	43.58±.08	1.17±.04
$18F_4H_{55}P_{16}P_{45}P_{82}$	48	..	..	..	..	..	1	1	8	20	26	10	3	1	..	..	..	..	..	70	43.66±.10	1.19±.07
$20.089(F_6 \times 18F_4H_{55}P_{16}P_{16}P_{51}P_{85})$	46	..	..	..	..	..	..	..	..	..	..	1	4	7	14	22	32	15	3	98	49.28±.10	1.45±.07
$21.068(F_6 \times 20.089P_{74})$	48	..	..	..	..	..	..	..	..	..	2	9	8	12	15	3	1	..	..	50	46.84±.13	1.39±.09

*Not included in computations.

There are four other F_4 populations in this table which represent progenies of immediate segregants from enlarged. $18F_4H_{55}P_{16}P_2P_{13}$ and $18F_4H_{55}P_{16}P_2P_{30}$ are progenies of normal segregants from $17F_3H_{55}P_{16}P_2$, the distribution for which is contained in table 4. There is a question as to the propriety of including the first of these populations in this table, because through some error measurements were not recorded of the parent plant. $18F_4H_{55}P_{16}P_{11}P_{39}$ is the progeny of a normal segregant of $17F_3H_{55}P_{16}P_{11}$, and $18F_4H_{55}P_{16}P_{21}P_{31}$ is the progeny of a normal plant from $17F_3H_{55}P_{16}P_{21}$. The two remaining populations indicate the behavior of normal segregants in F_5 and F_6. There are twelve populations in this table, all of which are of the normal type of frequency distribution. Other populations of normal segregants from enlarged plants have since been grown, and they likewise have given normal frequency distributions. It seems, therefore, established beyond reasonable doubt from the data presented in tables 2 and 3, that normal segregants from enlarged exhibit the same type of behavior as that characteristic of the *auriculata-macrophylla* cross. They breed true for the normal condition as contrasted with enlarged, to be described subsequently. Nevertheless, the populations exhibited segregation for minor size differences which may separate races differing as much in mean tube length as enlarged differs from normal; but the way in which they are established is that characteristic of multiple-factor systems.

THE ESTABLISHMENT OF ENLARGED

The distribution for tube length of the progeny of the original enlarged plant is recorded in table 4 under the caption $16F_2H_{55}P_{16}$. The range is obviously bimodal or it may even be trimodal, although the significance of these features of the distribution is not fully established on account of the small number of plants in the population. From this population a number of F_3 selections were made. The normal ones are included in table 3 and have been discussed in that connection. Four selections of enlarged plants were made and grown in F_3, and their distributions are entered in table 4 under the next four captions. When contrasted with corresponding normal F_3's in tables 2 and 3, there remains no question as to the genetic significance of the increased range of the enlarged F_2 distribution; but it is impossible to determine the precise nature of the difference because the behavior is not the same in the four F_3 populations. In particular $17F_3H_{55}P_{16}P_{16}$ is distinguished by the production of one individual with a tube length of 68 mm. Selfed seed was secured from this particular plant, and the F_4 progeny of it appears under the caption, $18F_4$

$H_{55}P_{16}P_{16}P_{51}$, in the table. Its distribution is characterized by the presence of a scattering group of plants exceeding 60 mm in tube length in addition to the normal and enlarged groups characteristic of other populations. These exceptionally large-flowered individuals we distinguish as "super-enlarged," because, as will become clearer from the discussion which follows, they differ not only in measurements but also in genetic behavior from those which we have called enlarged. Of the other four F_4 populations, $18F_4H_{55}P_{16}P_2P_{14}$ is difficult to interpret. There are clearly two groups of plants; but, if they are normal and enlarged, the proportions are reversed as compared with other populations. The other three F_4 populations exhibit two fairly sharply defined groups as shown more clearly by the correlation tables of length with spread than from length distributions alone.

From $18F_4H_{55}P_{16}P_{16}P_{51}$ two selections were made, one of an enlarged plant and one of a super-enlarged individual, and their progenies are included under the captions 20.090 and 20.092, respectively. 20.090 exhibits the two expected groups, normal and enlarged; and also contains one super-enlarged individual. 20.092, however, gave erratic results which cannot be satisfactorily interpreted even by reference to the correlation table. The discussion of super-enlarged contains further comments on its behavior.

TABLE 5

Results of self-fertilization of enlarged plants.

GARDEN NUMBERS	NORMAL	ENLARGED	TOTAL	PERCENT ENLARGED
$17F_3H_{55}P_{16}P_2$	66	30	96	31.3
$17F_3H_{55}P_{16}P_{11}$	82	16	98	16.3
$17F_3H_{55}P_{16}P_{21}$	36	13	49	26.5
$18F_4H_{55}P_{16}P_{11}P_{40}$	43	56	99	56.6
$18F_4H_{55}P_{16}P_{16}P_{53}$	47	46	93	49.5
$18F_4H_{55}P_{16}P_{21}P_{24}$	36	59	95	62.1
20.090	66	31	97	32.0
22.083	27	23	50	46.0
23.099	25	23	48	47.9
Total	428	297	725	41.0

Finally 22.083 is the selfed progeny of an enlarged plant of 21.065 (cf. table 9) and 23.099 is the selfed F_8 progeny of an enlarged plant of 22.083. These later populations exhibit a more definite separation of the two classes such as we would expect to get when the difficulty due to the segregation

TABLE 4

Distribution of tube length in progenies of selfed enlarged plants.

| GARDEN NUMBERS | PARENTAL VALUE IN MILLIMETERS | TUBE LENGTH IN MILLIMETERS | | |

of minor size differences has been eliminated. By successive generations of self-fertilization we have obviously succeeded in establishing an enlarged line which exhibits perfectly distinct segregation into normal and enlarged in its selfed progeny. It has not been possible to produce a race which breeds true for the enlarged condition.

The proportion of enlarged progeny produced by self-fertilizing enlarged plants is about forty percent. It varies, however, within rather wide limits as may be seen by reference to table 5, which contains a resumé of the data from table 4 of those populations which exhibited typical, unmistakable segregation for enlarged and normal. The variation in ratio of transmission is not, however, surprising, in view of the data presented below of transmission through pollen grains and ovules.

GAMETIC RATIOS OF TRANSMISSION OF ENLARGED

Having reduced the enlarged race to a condition of residual genetic homogeneity such that a dependable separation of enlarged and normal individuals could be secured, we set about the determination of transmission ratios through ovules and pollen in order to arrive at a satisfactory solution of the mode of transmission of the character.

In order to determine the ratios of transmission through ovules, a number of enlarged plants in 21.065 (table 9) were used as female parents in crosses with normal *N. sylvestris* and certain *Tabacum* races. The last were used in order to introduce other characters, so that the relation of segregation in these to enlarged might be determined. The parentage of the different populations and the number of normal and enlarged progeny are given in table 6, and the tube length distributions are contained in table 7. A word of comment concerning population 22.085 is, perhaps, necessary. This is an F_1 interspecific hybrid in which flower size is increased along with all other characters. It is interesting to observe, however, that the distinction between the two classes is even more sharply defined than in the intervarietal hybrids. Table 6 shows that the ratio of transmission through ovules varies within rather wide limits, from 20.8 to 44.9 percent, with a mean value of 35.5 percent. Table 7 shows that the separation of normal and enlarged groups is sharply discontinuous. In the few cases where intermediate individuals occur their proper classification was easily determinable from the correlation tables.

The transmission of enlarged through pollen grains was determined by growing progenies of normal ♀ × enlarged ♂. The distributions of these populations are contained in table 8. Of the six populations included therein, 22.086 represents a cross of *N. sylvestris* ♀ × enlarged ♂ from 21.065

TABLE 6

Results of crosses of the type, enlarged ♀ × normal ♂, to determine ratio of transmission through ovules

GARDEN NUMBERS	PARENTAGE	NORMAL	ENLARGED	TOTAL	PERCENT ENLARGED
22.084	$21.065P_{29}$ ♀ × $21.068P_{5}$ (*normal*) ♂	31	18	49	36.7
22.085	$21.065P_{29}$ ♀ × $21.050P_{22}$ (*sylvestris*) ♂	29	21	50	42.0
22.117	$21.065P_{25}$ ♀ × $21.019P_{16}$ (*apetala*) ♂	33	16	49	32.7
22.118	$21.065P_{27}$ ♀ × $21.015P_{1}$ (*alba*) ♂	38	10	48	20.8
22.119	$21.065P_{29}$ ♀ × $21.035P_{4}$ (*calycina*) ♂	30	20	50	40.0
22.120	$21.065P_{27}$ ♀ × $21.027P_{8}$ (*angustifolia*) ♂	38	12	50	24.0
22.132	$21.065P_{25}$ ♀ × $21.047P_{3}$ (*sessilifolia*) ♂	28	21	49	42.9
22.133	$21.065P_{27}$ ♀ × $21.014P_{1}$ (*purpurea*) ♂	27	22	49	44.9
Total		254	140	394	35.5

(cf. table 9), and the remaining five populations represent five different crosses of the type normal ♀ × enlarged ♂ made between two plants of 22.083 (cf. table 4). Among 298 plants only ten, or 3.4 percent, were of the enlarged type. The six populations uniformly exhibit a low proportion of enlarged; in fact, in one population there are no enlarged plants. The distinctive behavior of ovules and pollen grains in the transmission of enlarged is obviously closely parallel to that which BLAKESLEE (1921b) and his associates have found to be characteristic of trisomic characters in Datura.

SUPER-ENLARGED

As we have pointed out above, it seems to be characteristic of enlarged that on self-fertilization it produces an occasional extremely large-flowered individual which we have designated super-enlarged. If the ratio of transmission which we have determined separately for ovules and pollen grains, 35.5 and 3.4 percent, respectively, holds in self-fertilization, then we should expect the progeny of a self-fertilized enlarged individual to be in the ratio of 62.3 normal : 36.5 enlarged : 1.2 percent of super-enlarged individuals. It is natural, therefore, to assume that these super-enlarged individuals are the tetrasomic forms corresponding to the trisomic, enlarged, although no cytological confirmation of this supposition has been obtained.

That super-enlarged differs genetically from enlarged is established by the results which have been secured from certain crosses of the type, super-enlarged ♀ × normal ♂, and the reciprocal. Of the former type of cross, we have 21.065 = 20.090 P_{28} (super-enlarged) ♀ × 20.089 P_{74} (nor-

TABLE 7

Distributions for tube length in crosses of the type, enlarged ♀ × normal ♂. (For details of parentage see table 6)

GARDEN NUMBERS	TUBE LENGTH IN MILLIMETERS 42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	TOTAL
22.084	..	..	..	3	2	12	11	2	1	..	1	..	2	5	6	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	49
22.085	..	..	..	..	..	..	..	..	..	..	..	..	..	1	6	8	7	2	2	3	..	..	..	..	..	1	3	4	7	1	4	1	50
22.117	..	..	1	3	5	13	9	2	..	..	..	..	4	2	3	3	3	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	49
22.118	1	1	3	15	12	5	1	..	..	2	3	1	2	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	48
22.119	..	1	3	2	8	8	7	1	..	1	..	4	5	3	2	4	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	50
22.120	..	..	..	..	1	..	1	4	19	6	4	2	..	1	..	..	3	2	3	4	..	..	..	..	..	..	..	..	..	..	..	..	50
22.132	..	..	..	..	..	..	1	5	12	10	..	..	..	..	5	5	7	4	..	..	..	..	..	..	..	..	..	..	..	..	..	..	49
22.133	..	..	..	..	..	..	1	3	7	12	4	..	..	..	..	1	7	11	3	..	..	..	..	..	..	..	..	..	..	..	..	..	49

TABLE 8

Distributions for tube length in crosses of the type, normal ♀ × enlarged ♂.

GARDEN NUMBERS	TUBE LENGTH IN MILLIMETERS 45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	TOTAL
22.086	..	..	..	..	..	..	..	..	..	..	..	..	6	14	14	14	..	..	..	..	..	..	..	1	1	50
23.105	1	1	9	16	16	2	2	..	..	..	..	..	..	2	1	..	..	..	..	..	..	..	..	..	..	50
23.106	1	4	5	16	14	6	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	49
23.107	3	3	5	11	12	11	2	1	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	50
23.108	3	5	6	15	16	2	1	..	..	..	1	..	..	1	..	..	..	..	..	..	..	..	..	..	..	50
23.109	..	1	5	12	15	9	6	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	49

mal) ♂, and 21.067 = the same ♀ × *N. sylvestris* ♂. Of a total of 99 plants in the two populations 93 belonged to the enlarged group, and only 6 to the normal. The ratio of transmission of enlarged by super-enlarged through ovules amounts to 93.9 percent. 21.066 is the reciprocal of 21.065, the same individuals being used as parents. This population consisted of 24 normal and 26 enlarged plants, which indicates that about 52 percent of the pollen grains effective in fertilization bear an extra chromosome. The distributions are contained in table 9. The remaining four populations in this table were grown from four different capsules of seed secured by hand-pollination of flowers of $20.090P_{28}$, the super-enlarged parent of the three previous populations. They exhibit a surprisingly erratic behavior, but in the main the results are a confirmation of expectation based on the gametic ratios determined from the previous populations. If the ovule ratio is 94 percent enlarged + 6 percent normal, and the pollen ratio 52 percent enlarged + 48 percent normal we should expect to obtain a ratio of approximately 49 percent super-enlarged + 48 percent enlarged + 3 percent normal, among the progeny of a selfed super-enlarged plant. The actual distribution obtained among the 196 plants of the four populations is 20 percent super-enlarged + 77 percent enlarged + 3 percent normal. The enlarged class gains at the expense of super-enlarged; the normal class is present in about the expected ratio. It is interesting to observe that one population, 23.102, exhibits a distribution closely in agreement with expectation, whilst another, 23.101, contains no super-enlarged plants whatever. A tentative explanation for these discrepancies is offered in the discussion which follows.

The results with super-enlarged are provisional and should be extended. At least two of the populations in table 4, $18F_4H_{55}P_{16}P_{16}P_{51}$ and 22.092, are progenies of super-enlarged plants, but in both cases the classes intergrade to such an extent as to prevent accurate separation. It has been found that intergrading of enlarged and super-enlarged depends upon variation in the sizes of different flowers on the same plant. This is particularly true of super-enlarged plants. Flowers representing different periods in the development of a plant or its branches may differ rather strikingly in measurements, but when doubtful plants are kept under observation, and additional measurements are secured, it becomes possible to determine their correct classification without any difficulty. This fact was not recognized at first, but its application in the populations recorded in table 9 removed all difficulties from this source.

TABLE 9

Genetic behavior of super-enlarged. (For details cf. text.)

GARDEN NUMBERS	TUBE LENGTH IN MILLIMETERS																												TOTAL
	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	
21.065	..	1	1	1	..	..	..	..	5	10	16	14	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	49
21.066	1	3	7	7	6	..	..	..	3	3	6	11	2	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	50
21.067	..	..	..	..	..	..	..	..	..	..	..	..	1	1	1	..	..	..	..	..	..	..	..	5	12	16	13	..	1	50
23.100	..	..	..	..	..	..	..	2	3	7	11	8	7	1	..	..	2	..	4	2	..	2	..	..	..	..	..	..	..	49
23.101	..	..	..	..	1	..	1	..	..	6	12	9	4	12	4	..	..	..	..	..	..	..	..	..	..	..	..	..	..	49
23.102	..	..	..	..	..	..	..	..	..	1	2	5	7	9	1	..	..	3	4	8	5	2	2	..	..	..	..	..	..	49
23.103	..	..	..	..	2	1	..	..	..	4	7	7	8	12	2	..	..	..	..	1	4	1	..	..	..	..	..	..	..	49

DISCUSSION OF RESULTS

There is little necessity for an extended discussion of the results which have been presented in the foregoing portions of this paper. In the main they merely represent an extension to another species of the phenomena of trisomic inheritance.

The investigations of BLAKESLEE (1921 b) and his associates show that a trisomic character in Datura is transmitted on self-fertilization to about 25 percent of its progeny, and that transmission is mainly through ovules. Thus for the trisomic mutant, "globe," BLAKESLEE (1921 a) reports that 26 percent of ovules belong to the $(n + 1)$ class, as compared with only 2 percent of effective pollen grains. In Oenothera, in which a number of trisomic forms have been discovered, DE VRIES and BOEDIJN (1923) report that transmission is confined entirely to ovules. For enlarged in *N. Tabacum* the results are in the main in agreement with those from Datura, except that the proportion of $(n+1)$ ovules and pollen grains is somewhat higher, which leads to a higher transmission ratio on self-fertilization. This difference may be due to the fact that *N. Tabacum* has 24 pairs of chromosomes, instead of 12 as in *D. Stramonium*, so that the addition of an extra chromosome to a gamete might be expected to produce a less profound disturbance in balance and in functioning in the former species. This notion is likewise in agreement with the observation that enlarged differs less strikingly from normal than trisomic characters in Datura.

We should expect with a single extra chromosome that 50 percent of gametes would be of the $(n+1)$ and the remaining 50 of the (n) type. BUCHHOLZ and BLAKESLEE (1922) have presented evidence that in crosses of the type, globe ♀ × normal ♂, about 29.5 percent of ovules are aborted. If these are mostly $(2n + 1)$ zygotes, the gametic ratio may be very close to expectation. The discrepancy in the pollen ratio is more pronounced, and the same authors have presented evidence that this is probably largely accounted for by a slower growth rate of pollen tubes from $(n + 1)$ gametes, with a further elimination of a certain portion of the $(2n + 1)$ zygotes. Evidently similar phenomena occur in *N. Tabacum*.

In the matter of the genetics of the tetrasomic, super-enlarged, we appear to have no comparable cases in the literature. BLAKESLEE (1922) reports that round-leaf globe $(2n + 2)$ can be recognized by its accentuation of globe characters, and he presents photographs of young plants and capsules, but no data on genetic behavior. Super-enlarged in general bears the same relation to enlarged as round-leaf globe to globe; i.e., the tetrasomic is an accentuated expression of the trisomic. Its genetic be-

havior leads to important conclusions. We might think that tetrasomic forms should breed true, through production of $(n + 1)$ gametes only. This expectation is not, however, realized, for we actually find an ovule ratio of about 94 percent $(n + 1)$ and 6 percent (n), and an effective pollen ratio of 52 percent $(n + 1)$ and 48 percent (n). Here again the difference in ratio of transmission in ovules and pollen probably depends upon the same type of effect as that which is responsible for the difference in the trisomic. If we assume that the ovule ratio most nearly approaches the actual gametic ratio, then evidently 90 percent of reductions in the quadrivalent chromosome set are of the 2-2 type and 10 percent of the 1-3 type. The production of $(n + 2)$ gametes should result from the latter, but they have not been demonstrated. Possibly they are non-functional. If similar results in general follow the establishment of the $(2n + 2)$ condition, then obviously non-disjunction is excluded as a method of permanent increase in chromosome number. The genetic behavior of the trisomic and tetrasomic forms which have been thus far investigated would soon, apart from any selection against them, eliminate them from the population under natural conditions.

If an individual quadrivalent does not invariably exhibit the 2-2 type of reduction, then it would seem reasonable to search for some other basis for the constancy of tetraploids. It may be possible that they breed true because gametes and zygotes having an unbalanced constitution are eliminated in some way. In this connection we note that a large number of modified tetraploids have been recognized in Datura (cf. DAVENPORT 1923, p. 97), and BELLING and BLAKESLEE (1923) have shown from cytological studies that individual quadrivalents undergo 1-3 reduction in about 3 percent of cases.

The irregularity in behavior of super-enlarged as shown by the four populations in table 9 is probably to be accounted for by abortion of ovules and differences in growth rate of pollen tubes. The normal ovule ratio appears to be about 94 percent with an extra chromosome to 6 percent with the haploid number. If the pollen grains are produced in the same ratio we are forced to account for the high ratio of effective normal grains by a differential growth rate down the long style. Under the usual conditions of pollination, great variation undoubtedly occurs in the amount of pollen which is applied to the stigma. With a large excess of pollen, it is possible for a higher percentage of normal grains to become effective than when lesser amounts of pollen are applied, and the longer style of super-enlarged blossoms may enhance the chance of securing such discrepancies. We have found that super-enlarged is not at all easy to work with. Nor-

mally in *N. Tabacum* it is merely necessary to bag a portion of the inflorescence in order to secure selfed seed. With super-enlarged this procedure is rarely successful. Most of the flowers are abscissed without fertilization, and setting of seed can only be insured by hand-pollination. Crosses of super-enlarged ♀ × normal ♂ are more often successful than hand-pollination of super-enlarged, and crosses of normal ♀ × super-enlarged ♂ very commonly fail, or when they do succeed, they yield capsules only partially filled with seed. These results in a way support the reasons which we have presented for the erratic behavior of super-enlarged when self-fertilized.

SUMMARY

1. Enlarged is a trisomic character, depending on the presence of an extra chromosome.

2. Enlarged, on self-fertilization, produces about 41.0 percent of enlarged and 59.0 percent of normal plants, and an occasional super-enlarged individual.

3. Normal segregants from enlarged breed true for the normal condition.

4. About 35.5 percent of the functional ovules of enlarged plants transmit the enlarged character.

5. About 3.4 percent of the pollen grains of enlarged plants transmit the enlarged character, under normal conditions of pollination.

6. The genetic evidence indicates that super-enlarged is the tetrasomic condition corresponding to enlarged.

7. Self-fertilization of super-enlarged gives erratic results, but on the average 20 percent of the progeny are super-enlarged; 77 percent, enlarged; and 3 percent, normal.

8. About 94 percent of ovules of super-enlarged transmit the enlarged character, and 6 percent the normal.

9. About 52 percent of the pollen grains of super-enlarged transmit the enlarged condition and 48 percent the normal.

10. It is pointed out that these results are incompatible with the idea that permanent increase in chromosome number may result from nondisjunction.

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